at which ovulation may occur to the tenth day. On the basis of the recovery of these three unfertilized tubal ova, the time of ovulation in the menstrual cycle may be placed between the tenth and fourteenth days.

2986

The ovarian follicular hormone: a study of variation in pig, cow and human ovaries.

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It is now possible to measure quite accurately the amount of follicular hormone present in the ovaries of the larger mammals. The unit of measurement is the least amount of lipoid extract required to produce a definite physiological reaction in the spayed adult rat. This reaction to the injected hormone proves a good physiologic unit of measurement because it has a fairly sharp end point which may be determined accurately without sacrificing the test animal. It has been fully described and illustrated in earlier papers.[1] Briefly stated, the test consists of the induction of enough growth in the genital tract to cause the formation of a cornified layer in the vaginal epithelium. This growth requires about 48 hours and amounts to a complete replacement of the epithelial wall of the vagina. A positive test is indicated when cornified cells replace the leucocytes typical of the vaginal smear of the spayed control animal.

Material from pigs accurately timed with regard to its position in the 21 day oestrous cycle was collected by Dr. F. F. McKenzie, Department of Animal Husbandry, University of Missouri, in the course of his studies on reproduction.[2] The first ovary ex-

[1] (a) Allen, Edgar, and Doisy, E. A., *J. Am. Med. Assn.*, 1923, lxxxi, 819. (b) Allen, Edgar, Doisy, E. A., Francis, B. F., Robertson, L. L., Colgate, C. E., Johnston, C. G., Kountz, W. B., and Gibson, H. V., *Am. J. Anat.*, 1924, xxxiv, 133. (c) Doisy, E. A., Rolls, J. C., Allen, Edgar, and Johnston, C. G., *J. Biol. Chem.*, 1924, lxi, 711. (d) Allen, Edgar, and Doisy, E. A., *Am. J. Physiol.*, 1924, lxix, 577.

[2] McKenzie, Fred F., *Anat. Rec.*, 1924, xxvii, 185.

tracted was from a sow killed on the 19th day of the oestrous cycle, just as external signs of sexual activity were again appearing. This ovary weighed 7 grams and contained 9 large follicles of 10.5 mm. average diameter. The follicles were slit open and the whole ovary extracted with three changes of several times its volume of 95 per cent alcohol. The residue from evaporation of this extract was dissolved in corn oil. This solution, made up in a graded series of dilutions, was then tested by injection into spayed rats as outlined above. Results of these tests returned a total of 15 rat units of hormone.

The other ovary from this animal contained 8 large follicles of the same average size. Consequently the two ovaries were quite equally sharing function at this oestrous. The hormone content of the second ovary may therefore be estimated at about 13 rat units. This would make a total of 28 rat units for both ovaries of this sow on the first day of oestrus. It should be noted in this connection, however, that the production of 17 follicles at one oestrus in the pig, though not unusual, is more than the average number.

An ovary from another animal of this series was removed on the second day following the first appearance of external signs of oestrus. Ovulation had occurred and eleven ova were recovered from this one oviduct. There were eleven *corpora lutea* of the first or second day of development in this ovary. Tests of the extract were negative in doses up to an equivalent of one-half the total extract. The second ovary from this animal contained only one *corpus luteum,* consequently the ovary extracted was bearing practically all the function at this oestrous period. An estimation of the total hormone content of both ovaries would therefore be less than 2.1 rat units. Low yields have also been obtained from pig ovaries removed as late as one week before oestrus, but further tests must be made of these stages.

These results indicate a variation in the follicular hormone content from more than 28.0 rat units shortly before ovulation to less than 2.1 units shortly after ovulation in the ovaries of these two sows.

The cow material extracted was collected by Drs. H. S. Murphey and G. W. McNutt, Department of Veterinary Anatomy, Iowa State University. It was not possible to place the ovaries in accurate time relations in the cycle by observations on the animals before killing. The ovaries were grouped, however, by

correlating the condition of the genital tract and the visible ovarian structures with earlier results from observations upon normal animals.[3] These groups represented five different intervals of the 21 day oestrous cycle: (1) "in oestrus," (2) 1 to 5 days, (3) 5 to 10 days, (4) 10 to 17 days, and (5) 17 to 20 days after oestrus. Each group consisted of from 20 to 30 ovaries, both ovaries from each cow being included. Before the second extraction the ovaries were cut into thin sections and the follicles and *corpora lutea* counted and measured.

The extract of the group of 26 ovaries taken at oestrus, when the follicles were largest, returned a total of 60 rat units of hormone, or an average of 4.6 units per cow. It is considered that this represents the average minimum present in one large follicle 1 or 2 days before ovulation, for except in cases of dizygotic twins the cow usually ovulates only one ovum at each oestrus.

Extracts of the other four groups of cow ovaries each returned negative results in portions as large as one-third the total extract of that group. This means the presence of only very small amounts of follicular hormone in the ovaries of the cow at times other than oestrus. It should be noted in this connection that each of these four groups of cow ovaries contained from 8 to 11 large *corpora lutea* covering time intervals up to 21 days of development. It would seem, therefore, that the *corpus luteum* of the cow, like that of the pig, contains very little if any of the follicular hormone.

For purposes of comparison with these results reference is made to work done in collaboration with Drs. J. P. Pratt and E. A. Doisy in measuring the amount of follicular hormone in human ovarian tissues.[4] Briefly summarized: (1) The highest yield of hormone from human liquor folliculi was 7 rat units per cc. (2) The highest yield from human corpora was obtained from those removed at operation during the third week following the first day of the previous menstruation. This amounted to 3.7 rat units per cc. of luteal tissue (the volume of the corpora being measured by the amount of fluid displaced by the tissue after the first extraction. (3) Corpora removed during the fourth week of the menstrual cycle yielded 2 rat units per cc.

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[3] (a) Murphey, H. S., *J. A. V. M. A.*, 1924, viii (n. s.), 598. (b) McNutt, G. W., *J. A. V. M. A.*, 1924, xviii (n. s.), 556.

[4] Allen, Edgar, Pratt, J. P., and Doisy, E. A., *J. Am. Med. Assn.*, 1925, xcv, 399.

A summary of our analyses of ovarian tissues of these three mammals brings out several main points. (1) In the ovaries of cows and pigs there is a wide variation in the amount of follicular hormone present at different times in the cycle, a variation directly correlated with the size of the follicles. (2) The maximum attained during oestrus drops rapidly at about the time of ovulation and the newly forming corpora contain very little of this active substance. (3) Analyses of human ovarian tissues, besides showing a high hormone content in liquor folliculi, show also a considerable amount of the follicular hormone in the *corpus luteum.*

It has been assumed by most workers in this field that the endocrine function of mammalian ovaries is the same regardless of the species. There seem to be few differences in the formation of follicles and maturation of ova that would suggest otherwise. Anatomical differences have been described in the transition of follicles to *corpora lutea* in human beings and other mammals, but no functional discrimination has as yet been made among corpora which is typical of any group or species. The difference shown in our results may prove significant in explaining the differences in sexual phenomena in primates and in the lower mammals.

Review of the recent literature[5] discloses several differences between the menstrual cycle of primates and the oestrous cycle of pigs and cows. (1) The evidence for primates indicates that ovulation occurs in the intermenstrum. In the lower mammals it occurs toward the end of eostrus. (2) The oestrous cycle in the lower mammals is characterized by a short period at about the time of ovulation to which mating instincts are normally restricted, while in the primates studied the mating instincts may be diffused over practically all of the menstrual cycle. (3) The duration of the cycle in primates is a week longer than in the pig and cow. (4) The catabolic phase in the genital tract, whether it be hemorrhage or leucocytic infiltration, more closely follows ovulation in the oestrus than in the menstrual cycle.

The ovarian follicular hormone exerts a growth inducing influence upon the genital tract which raises it to maximum function including secretory activity of the glandular epithelium. It

[5] (a) Marshall, F. H. A., The Physiology of Reproduction, London, 1922; *Physiol. Rev.*, 1923, ii, 355. (b) Corner, G. W., *Physiol. Rev.*, 1923, iii, 457. (c) Murphey, H. A., McNutt, G. W., Zupp, B. A., and Aitken, W. A., *J. A. V. M. A.*, 1925, lxvii, 1.

also bears a causal relationship to mating instincts.[1b] Consequently the rapid increase in the reserve supply of this hormone in the ovaries of pigs and cows to a maximum at oestrus would result in a rise of the growth curve of the genital tract and also in a climax of mating instincts. The rapid drop following ovulation would remove this stimulus and allow the early succession of the degenerative phase. In the primates on the other hand, the high concentration of this hormone in the follicle, probably rising to a maximum during the latter part of the second week, and continuing in relatively high concentration in the *corpus luteum,* would extend considerably the period of influence of this hormone. This might account in part for: (1) the longer duration of the menstrual cycle, (2) the longer portion of the cycle during which mating instincts may be in evidence, and (3) the continuance of the anabolic and secretory phase for a considerable period after ovulation.