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Ovarian Steroid Dehydrogenase Histochemistry and Circulating Progesterone in Aged Golden Hamsters During the Estrous Cycle and Pregnancy[1]

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The histochemical activity of the enzymes Δ5-3β-hydroxysteroid dehydrogenase (3βHSD) and 17β-hydroxysteroid dehydrogenase (17βHSD) was studied in the ovaries of aged golden hamsters. These enzymes are operative in progesterone synthesis (3βHSD) and 17β-estradiol–estrone interconversion (17βHSD). Blood levels of circulating progesterone were determined in the same animals. Data from the old animals were compared to measurements of these same parameters from young hamsters. The pattern of 3βHSD activity was similar in young and old animals, appearing in follicles and corpora lutea at appropriate times of their function and in interstitium at all times. The 17βHSD activity was only found in follicular granulosa cells during the cycle and the first half of pregnancy of young and old animals, but was also found in corpora lutea in later pregnancy. The ovaries from old animals differed from those from young animals mainly in the degree of development of various components. There was much individual variability, but often old ones had fewer large follicles, fewer ovulations, and more corpora lutea atretica. Interstitium with 3βHSD was less abundant and replaced by pigment or condensed inactive stroma. Circulating progesterone was not significantly lower than normal in old hamsters and on certain days some even had exceptionally high levels compared to young. Old hamsters carried fetuses to term but did not deliver them, apparently because their corpora lutea continued to secrete progesterone. The fact that many old hamsters resorb conceptuses or fail to have implantations does not appear to be primarily related to lack of progesterone or deficiency of ovarian steroid dehydrogenases.

Certain aspects of the senescent decline of reproductive ability in female golden hamsters are particularly notable. Of special interest is the prolongation of gestation as the age of the mother increases (Soderwall *et al.*, 1960). This prolonged gestation may result in the delivery of dead fetuses or failure of delivery followed by resorption (Blaha, 1964a). In a more advanced stage, there are many resorptions before term. Ultimately, in old females, blastocysts fail to implant even though mating and pseudopregnancy still occur (Blaha, 1964a; Thorneycroft and Soderwall, 1969). In the present study, old female hamsters were examined in two stages of reproductive decline: (1) those with prolonged gestation, and (2) those showing decreased implantations and/or early resorption. The ovarian condition was studied in each animal by histochemical methods for two enzymes. The first, Δ5-3β-hydroxysteroid dehydrogenase (3βHSD) operates in conversion of Δ5-pregnen-3β-ol-20-one (pregnenolone) to progesterone. The second enzyme, 17β-hydroxysteroid dehydrogenase (17βHSD), is reversibly active in the interconversion of 17β-estradiol and estrone (Talalay, 1965). Circulating levels of progesterone were measured in vena caval

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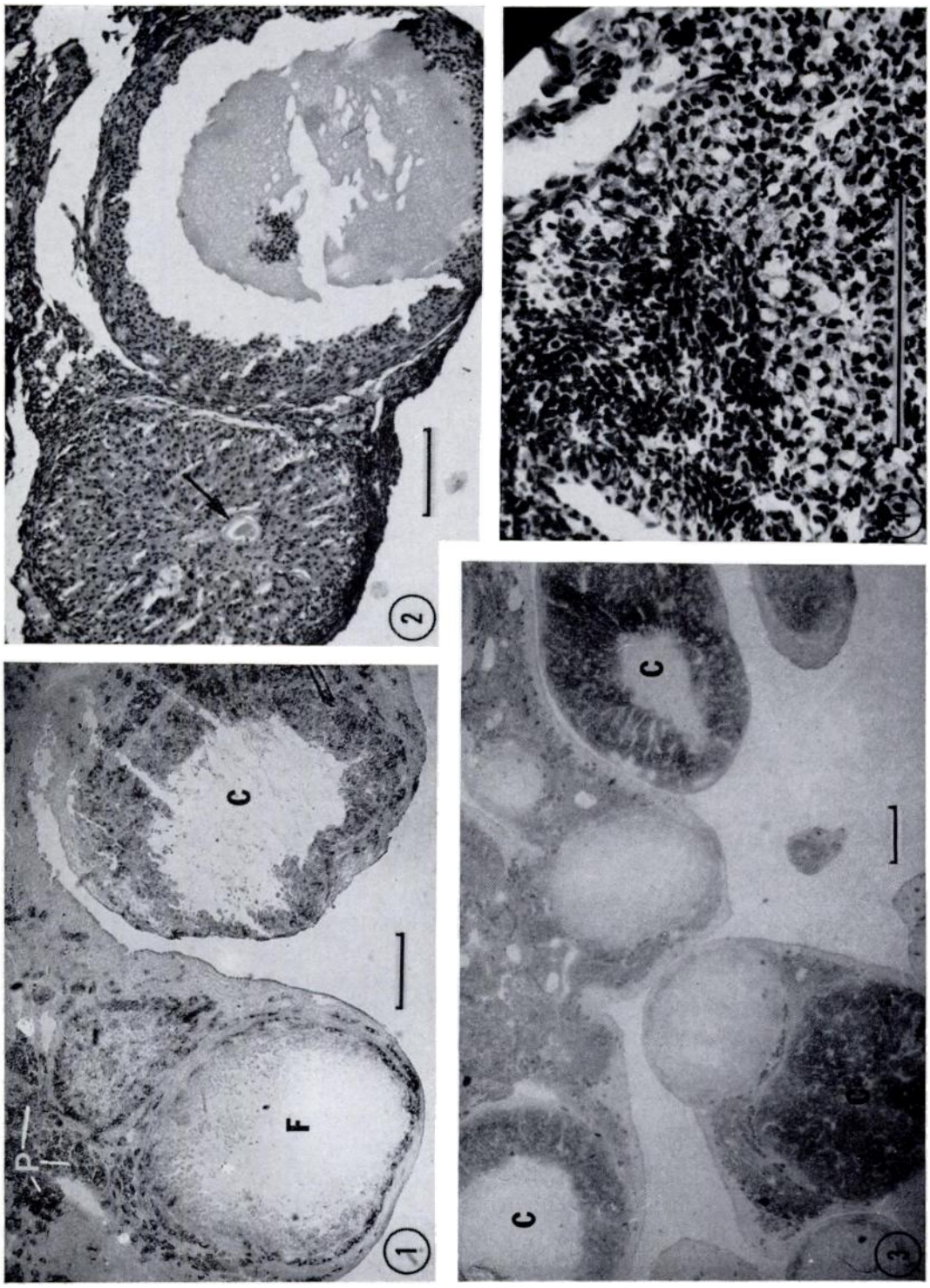
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blood and correlated with ovarian enzyme activity in each animal. These results were compared to similar measurements made in young hamsters, some of which have been published previously (Leavitt and Blaha, 1970; Blaha and Leavitt, 1970).

MATERIALS AND METHODS

Female golden hamsters were caged individually under conditions of controlled temperature (21–23°C) and lighting (lights on 2300–1200 h EST). Hamster chow and water were provided to choice and lettuce was given twice weekly. Estrous cycles were determined by the postovulatory vaginal discharge with several consecutive 4-day cycles being ascertained. Matings were in the early dark period 3–7 h before the expected time of ovulation. Mating was observed and the presence of sperm was checked after at least 20 min of mating. Day 1 of the cycle or pregnancy is the day following ovulation.

The age of the hamsters studied during prolonged gestation was 15–18 mo. Those studied during the cycle and early pregnancy were 17–23 mo old and were judged by previous breeding performance to have reached a stage where they were unlikely to carry young to term. Only old females which exhibited regular cycles and were capable of mating were used.

At autopsy, animals were anesthetized with sodium pentobarbital (9 mg/100 g ip). Before removal of ovaries, blood samples were taken from the posterior vena cava for analysis of progesterone content.

Histochemical analysis for ovarian 3βHSD and 17βHSD activity was carried out on each day of the estrous cycle and on Days 1–9, 16, 17, and 18 of pregnancy. Tissues from young animals (3–5 mo) served as controls and were as previously described (Blaha and Leavitt, 1970). The enzyme histochemistry was performed as in the same previous study with the addition that for 17βHSD, 17β-estradiol was used for the substrate and NADP was substituted for NAD as the cofactor unless stated otherwise. As before, the activity of 3βHSD was rated on a scale from 0 to 5, with control incubations (no substrate) having zero activity. Portions of ovaries not used for HSD study were fixed in formalin, embedded, and sectioned for histological study. Some of the frozen sections were also stained by standard histologic procedures. Uteri were examined macroscopically and histologically to determine the condition of implantation sites or fetuses.

Progesterone analysis. The plasma samples were obtained, processed, and analyzed for progesterone content by gas–liquid chromatography exactly as described previously (Leavitt and Blaha, 1970). All determinations were corrected for procedural losses, and the results are expressed according to the original blood volume (μg progesterone/100 ml blood). Student's *t* test was used to compare the data from old and young animals.

RESULTS

Histochemistry

The ovarian compartments containing 3βHSD activity in old females were the same as previously reported for young hamsters (Blaha and Leavitt, 1970). Activity of 3βHSD was localized in corpora lutea, interstitium, and follicles at the different times studied. As follicles grew during the cycle, the theca interna developed 3βHSD activity which reached a maximum (rated +5) in early estrus. Granulosa cells contained slight 3βHSD activity on Day 4 (+1—2) which increased after ovulation (+3) as the new corpora lutea formed (Fig. 1). The corpora lutea of the cycle had maximal 3βHSD activity (+5) on Day 2 (Fig. 3). Corpora lutea of mated

FIG. 1. Activity of 3βHSD in a 22-mo-old hamster, 15 h after ovulation. Activity is found in a new corpus luteum (c), and the theca interna of an atretic follicle (f). Pigment (p) is in the interstitium, as are also some cells with 3βHSD. The scale on all figures indicates 200 μm.

FIG. 2. H and E stain of an old ovary similar to Fig. 1, showing a corpus luteum (atreticum) with the oocyte remaining (arrow) and an unovulated follicle.

FIG. 3. Day 2 of the cycle at 21 mo. Full development of 3βHSD in a solid corpus luteum and two others with open centers (c). Follicles (f) show no activity and the interstitium has a normal level of 3βHSD.

FIG. 4. H and E stain of ovarian interstitium in a 21-mo-old hamster. The lighter, vesicular area at the bottom corresponds to an area of 3βHSD activity on adjacent sections. The area of densely packed nuclei at the top was inactive for 3βHSD.

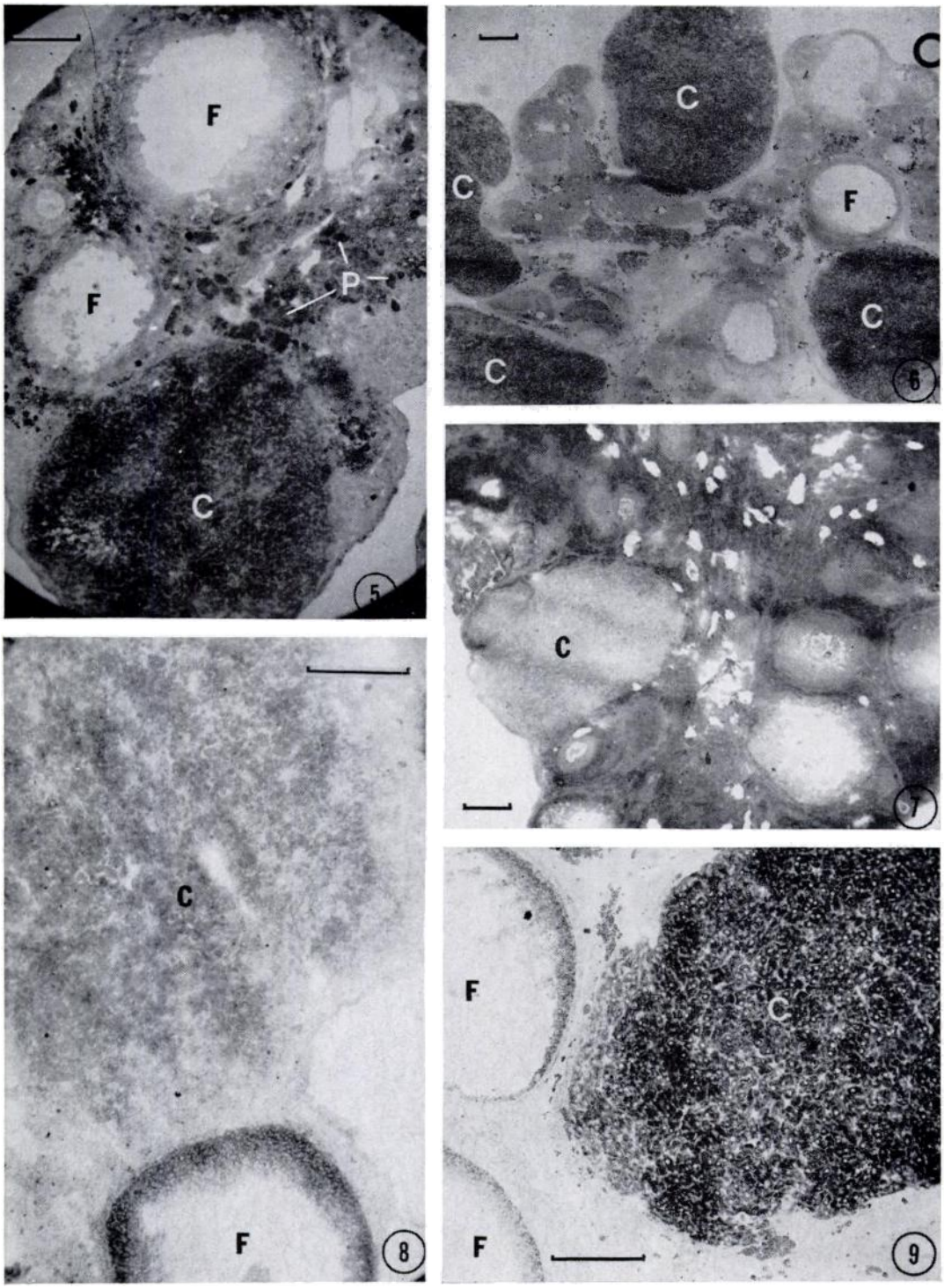

FIG. 5. Ovary of a 23-mo-old hamster 9 days after mating. No implantations were found. Large follicles of pregnancy (f) and much interstitial pigment (p) can be seen above the corpus luteum (c) which has strong 3βHSD activity. The scale on all figures equals 200 μm.

animals continued to show this level of 3βHSD on Day 3 and beyond (Fig. 5). Luteal 3βHSD activity was reduced by Day 3 in unmated animals and was virtually absent on Day 4.

The ovaries of old hamsters differed from young ones in several respects. There was greater individual variability in the extent of development of the ovarian components. In some, only one or a few follicles were found, although in most old animals follicle number was near normal. More frequently, some of the follicles failed to ovulate, resulting in abnormal corpora lutea (Figs. 1–3). This condition was usually not complete in any one animal, and atretic follicles with 3βHSD in the theca, partially luteinized follicles or corpora lutea atretica and normal corpora lutea were all found on Day 1. However, in most of the old females, a majority of follicles appeared to have ovulated. A strong 3βHSD reaction (+5) developed in all luteinized structures by Day 2 of the cycle (Fig. 3). Of interest in the ovaries of many old animals was an obvious reduction in the amount of interstitium showing 3βHSD activity. This was replaced by two other kinds of tissue, one pigmented and one not pigmented.

Accumulations of pigment-containing cells were common (Figs. 1, 5, and 6). These resembled macrophages and stained with PAS, Prussian blue, Perl's ferrocyanide reaction, and Oil red O. Nonpigmented interstitium which lacked 3βHSD activity had closely packed, dark nuclei when stained by hematoxylin and eosin (Fig. 4). This contrasted with the more abundant cytoplasm and lighter nuclei of interstitial cells which still contained 3βHSD activity in adjacent sections. As in the young female, interstitial 3βHSD activity varied little in intensity from day to day, although the number of active interstitial cells varied considerably from animal to animal.

The group with prolonged gestation differed from young females in regard to the timing of luteolysis. In the young hamster during pregnancy, histochemical activity of 3βHSD started to wane on Day 15 and was decreased notably on Day 16 before delivery (Fig. 7). Old hamsters with term fetuses had corpora lutea with intense 3βHSD activity on Day 16 (Fig. 6). This activity declined gradually on Days 17 and 18, to a level comparable to interstitial activity (+3). The number of fetuses in these old females ranged from one to nine, lower than the number in normal young females (range 8–16).

Using NADP as the cofactor, the histochemical activity of 17βHSD was only slight to moderate in the granulosa of follicles on Days 3 and 4 of the cycle and during the first half of pregnancy in both young and old hamsters. This activity increased in follicles and appeared in corpora lutea during the second half of gestation. With NAD as the cofactor, only luteal 17βHSD activity was found in the second half of pregnancy. In young hamsters, luteal 17βHSD faded as luteolysis occurred before parturition (Fig. 8), while follicular 17βHSD persisted until the large follicles regressed following parturition. In old hamsters with prolonged gestation, both ovarian compartments showed a continuation of 17βHSD activity into Day 16 (Fig. 9), and this decreased gradually on Days

FIG. 6. Ovary of a 17-mo-old hamster at 16 days of pregnancy, showing strong 3βHSD activity in the corpora lutea (c) and lesser activity in follicles (f) and interstitium.

FIG. 7. Ovary of a 3-mo-old hamster at 16 days of pregnancy, showing the normal prepartum loss of 3βHSD activity in the corpus luteum (c).

FIG. 8. Ovary of a young hamster at the beginning of delivery on Day 16. The 17βHSD activity in the corpus luteum (c) has decreased but 17βHSD is still apparent in the follicle (f).

FIG. 9. Same old ovary as Fig. 7, showing the activity of 17βHSD in the corpus luteum (c) as well as the granulosa of two follicles (f).

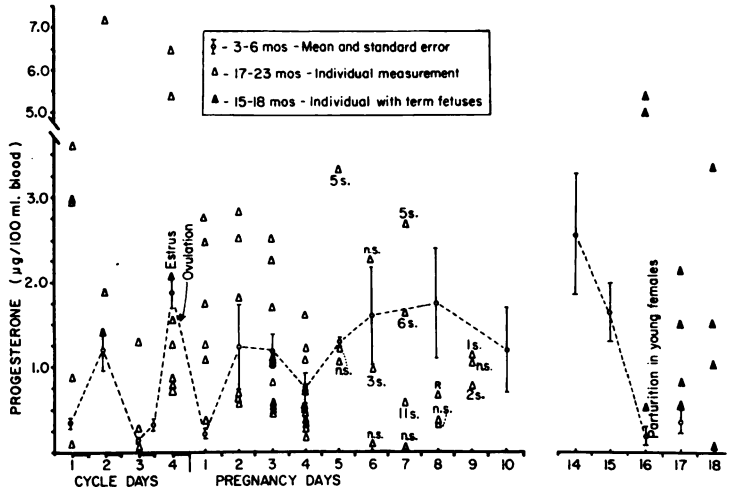

FIG. 10. Circulating progesterone in golden hamsters during the estrous cycle and pregnancy (s = implantation sites; ns = no implantation sites; R = Resorbing implantations).

17 and 18. No 17βHSD activity was seen in interstitium of young or old.

Progesterone Levels

Criteria for the identification and measurement of progesterone in vena caval blood were the same as previously described (Leavitt and Blaha, 1970). The levels determined in young individuals served as the basis of comparison (Fig. 10). Because there was great variability in the progesterone levels of old hamsters, the individual results are reported on the graph.

During the estrous cycle and pregnancy there were more instances of old hamsters with high progesterone levels than there were with low levels. Circulating progesterone levels were higher in old females on Day 1 of either the cycle or pregnancy ($p < 0.05$ for the cycle and $p < 0.005$ for pregnancy) when compared to low progesterone in the young females. Mean progesterone levels were in the normal range in old hamsters during the interval (Days 2–6) when changes should have been occurring in preparation for implantation. Although the mean levels in old hamsters were not statistically different from normal, many single values were well above or below the mean and standard error of progesterone levels in young hamsters. Most of the old females had few or no implantations on Days 5–9. Microscopic study of the implantation sites present showed many to be retarded or resorbing. Detectable, though low, levels of progesterone through Day 9 correlated with the presence of histochemically active corpora lutea.

DISCUSSION

The average length of gestation increases with maternal age in hamsters (Soderwall *et al.*, 1960). In our colony, the exact duration of prolonged pregnancies cannot be determined because either delivery does not occur or it may be extended and incomplete. Parturition in young hamsters normally follows the decline of progesterone secretion in a manner similar to that described in the rat (Csapo and Wiest, 1969; Leavitt and Blaha, 1970). The decline in the secretion of progesterone in hamsters appears to result from luteolysis between Days 15 and 16 (Blaha and Leavitt, 1970). Luteolysis is delayed in the old hamster with term fetuses and this may account for the extension of gestation, presumably as a result of the continuation of progesterone secretion. Other factors, such as estro-

gen, probably also play a role in parturition. As pointed out by Csapo and Wiest (1969) and as we have observed (unpublished observations), rats or hamsters ovariectomized near term do not have normal parturition. The 17βHSD found in large follicles and corpora lutea suggests either production or metabolism of estrogens in late pregnancy. Joshi and Labhsetwar (1972) measured very high levels of estradiol and estrone from midpregnancy to term in hamsters. The source of estrogen during the cycle is believed to be antral follicles (Baranczuck and Greenwald, 1973), but the 17βHSD in corpora lutea of pregnancy suggests these could contribute to the estrogen production from midterm to near term.

The cause of the prepartum luteolysis in normal hamsters is not known, and it is not clear why luteolysis is delayed in the aged female hamster. It is perhaps significant that the number of fetuses is less than normal in most old hamsters. A statistically significant inverse relationship between the number of fetuses and the length of gestation has been demonstrated in several species (Goy *et al.*, 1957; Biggers *et al.*, 1963). McLaren (1967) has presented evidence suggesting that the fetal mass is more important than the number of fetuses in determining the length of gestation. It appears from our study that control of luteal function could be the means by which these other factors exert their effects. Although fetal mass or number is important, there may be other factors which contribute to parturition delay in old hamsters as evidenced by old individuals with five to nine fetuses which were delayed beyond the normal time of delivery expected in young females.

The old hamsters examined in early pregnancy were taken at a time when they were unlikely to carry young to term, based on their age and previous breeding performance. The small number of implantations, many retarded or resorbing, indicated that most of these females were in an advanced state of reproductive decline. As reported by Thorneycroft and Soderwall (1969), there is a great preimplantation loss in old hamsters. It did not appear that lack of circulating progesterone would account for this result. When implantation should have been established during the first 6 days of pregnancy, mean levels of progesterone in these old animals were not significantly different than levels in young animals. However, some individuals did have very low levels which were compensated in the mean by other individuals with high levels. Optimal levels of circulating progesterone necessary for implantation are not known. Lower levels of progesterone were found from Days 6 to 9 in old females which had few or no implantations but had corpora lutea with 3βHSD activity. The declining luteal secretion would be more comparable to pseudopregnancy in these cases. Many ova in old hamsters are either not fertilized or for some reason do not reach the uterus (Blaha, 1974). Abnormal progesterone levels during the cycle before mating may have an adverse effect on pregnancy. Hunter (1968) found that progesterone treatment given to hamsters on Day 3 or 4 of the cycle lowered the fertilization rate, possibly because of altered sperm transport.

The progesterone levels on Day 1 of both the cycle and pregnancy in old hamsters averaged much higher than the levels which we observed in young hamsters at these times (Leavitt and Blaha, 1970). There was no histological evidence of excessive ovarian activity in the old hamsters. The failure of some follicles to ovulate and the accumulation of pigment could be associated with an alteration in progesterone secretion. It is also possible that progesterone metabolism is reduced in old hamsters, allowing the high preovulatory levels to be maintained into the postovulatory period. A reduced progesterone metabolism could account for higher levels of hormone found later in the cycle or pregnancy as well.

While some of the anovulatory corpora lutea had a structural resemblance to the "luteal bodies" in splenic ovarian grafts described by Leavitt *et al.* (1972), they did not persist beyond their normal times, following the same course as normal corpora lutea. Horowitz (1967) has described anovulatory corpora lutea in hamsters, but did not relate them to the age of the animals. Failure of ovulation, however, accounts for only a small percentage of the pregnancy loss.

The fact that progesterone is not deficient in most old females supports the conclusion that failure in early pregnancy, despite the presence of blastocysts (Blaha, 1964a,b), can be at least partly attributed to a defect in the uterine tissue. This conclusion is supported by the finding that old hamsters were less capable of developing deciduomata than were young ones (Blaha, 1967). Finn (1966) reported similar findings with old mice. The deficiency in the uterine tissue of aged hamsters might be either in the uptake (Larson *et al.*, 1972), metabolism, or uterine response to progesterone. The retarded development of implantation sites in old hamsters, as observed in this study and elsewhere (Connors *et al.*, 1972; Parkening and Soderwall, 1973), is suggestive of a reduced uterine responsiveness to the normal levels of progesterone which are available.

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