Journal of Gerontology
1978, Vol. 33, No. 2, 191-196

Circulating Plasma Levels of Pregnenolone, Progesterone, Estrogen, Luteinizing Hormone, and Follicle Stimulating Hormone in Young and Aged C57BL/6 Mice During Various Stages of Pregnancy[1]

Terry A. Parkening, PhD,[2,3] In-Fai Lau, PhD,[2]
Shiva K. Saksena, DVM,[2] and Min-Chueh Chang, PhD, ScD[2]

Young (3-5 mo of age) and senescent (12-15 mo of age) multiparous C57BL/6 mice were mated with young males (3-6 mo of age) and the numbers of preimplantation embryos and implantation sites determined on days 1 (day of plug), 4, 9, and 16 of pregnancy. The numbers of viable embryos were significantly lower ($p < 0.02$ to $p < 0.001$) in senescent females compared with young females on all days except day 1 of pregnancy. Plasma samples tested by radioimmunoassay indicated circulating estradiol-17B was significantly lower ($p < 0.05$) on day 1 and higher ($p < 0.05$) on day 4 in older females, whereas FSH was higher on days 4, 9, and 16 ($p < 0.02$ to $p < 0.001$) in senescent females when compared with samples from young females. Levels of pregnenolone, progesterone, estrone, and LH were not significantly different at any stage of pregnancy in the two age groups. From the hormonal data it did not appear that degenerating corpora lutea were responsible for the declining litter size in this strain of aged mouse.

IT IS well known that aging adversely affects the reproductive capacity of female mammals. This is exemplified in laboratory rodents by a progressively declining litter size. Such loss of fecundity is not due to a decline in the rate of ovulation but to preimplantation and postimplantation embryonic loss (Biggers et al., 1962; Harman & Talbert, 1970; Maibenco & Krehbiel, 1973; Parkening & Soderwall, 1973, 1974; Talbert & Krohn, 1966; Thorneycroft & Soderwall, 1969). While it is obvious that every organ undergoes changes during aging, the uterus is commonly considered to be the organ most responsible for a smaller litter size (Biggers, 1969; Finn, 1970; Larson et al., 1972; Shapiro & Talbert, 1974), although changes within the hypothalamic-pituitary complex (Labhsetwar, 1970; Peng & Peng, 1973; Watkins et al., 1975) and the ovary (Albrecht et al., 1975; Leathem & Shapiro, 1975) have also been implicated in this reduction. Recent examinations of the ovaries of aged mice have shown that malfunctioning corpora lutea may cause early embryonic death (Gosden, 1974, 1975; Harman & Talbert, 1970). While hormonal deficiencies or imbalances may exist in the aged female (Parkening, 1976; Parkening & Soderwall, 1975), the few hormonal studies conducted on senescent rodents during pregnancy have been restricted to rats (Howland & Preiss, 1975; Watkins et al., 1975) and hamsters (Blaha & Leavitt, 1974). This study was conducted to determine the circulating plasma levels of pregnenolone, progesterone, estrone, estradiol-17B, luteinizing hormone (LH), and follicle stimulating hormone (FSH) in young and aged C57BL/6 mice on days 1, 4, 9, and 16 of pregnancy in order to determine if differences exist in the hormonal levels of the two

[1]We would like to thank Dr. G. D. Niswender and Dr. A. R. Midgley, Jr., for LH antiserum; Dr. L. E. Reichert, Jr., for LH standards; and Miss C. Roberson for LH and FSH assays. This research was supported by a postdoctoral fellowship (1 F22 HD00716) to T. A. P. and a grant from NICHD (HD 03003).

[2]Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

[3]Present address: Dept. of Anatomy, Univ. of Texas Medical Branch, Galveston 77550.

age groups which may be correlated with a reduction in litter sizes in senescent females.

MATERIALS AND METHODS

Young (3-5 mo of age) and senescent (12-15 mo of age) multiparous C57BL/6 mice (Jackson Laboratory) were bred to young males (3-6 mo of age) of the same strain and killed on 1 (day of copulation plug), 4, 9, and 16 days of pregnancy. All of the young females and a majority of the aged females exhibited a normal 4-5 day estrous cycle prior to mating. All animals were maintained on a 14-hour light: 10-hour dark photo-period with lights on at 0500 hour (EST). Using a heparinized syringe with a 27-gauge needle, blood was obtained from unanesthetized females by cardiac puncture between 1000 and 1100 hours. Individual samples were placed in heparinized tubes, centrifuged at 1000 X *g* (4 C) for 15 min and stored at -20 C until assayed. The amount of plasma obtained from a single animal ranged from .35 to .9 ml, with the majority yielding a sufficient amount to assay steroids and one gonadotropin.

On day 1 the oviducts were excised, placed in Hanks' solution containing 0.05% hyaluronidase, and the ampullae dissected to release the eggs within the cumulus. After the dispersal of the cumulus and follicular cells, the eggs were counted and classified as normal or abnormal. Uterine horns were removed and flushed with Hanks' solution by means of a 27-gauge needle on day 4 of pregnancy and the blastocysts were counted and classified as normal or abnormal. On days 9 and 16 of pregnancy, implantation sites were counted and examined for viable and resorbed fetuses. Ovaries were removed from each female on days 4, 9, and 16 and the number of corpora lutea was counted under a dissecting microscope.

Assay for steroids. — Plasma pregnenolone, progesterone, estrone, and estradiol-17B concentrations were determined from 0.2-0.4 ml of plasma by separation on a micro-celite column (Purvis et al., 1975) and assayed by radioimmunoassay (Saksena et al., 1976). Tritiated pregnenolone, progesterone, estrone, and estradiol-17B (~2500 dpm in 0.1 ml of phosphate buffer, pH 7.4 for each steroid) were added for the determination of recovery losses. The average recoveries for pregnenolone, progesterone, estrone, and estradiol-17B were, respectively, 67%, 72%, 78%, and 62%; the intra-assay coefficients of variation were 8%, 8%, 10%, and 11%, respectively. All comparisons were based on samples measured in the same assay and water blanks tested did not exceed the sensitivity of the assay and were not subtracted from the values reported. The standard curves for pregnenolone, progesterone, estrone, and estradiol-17B ranged from 12.5-800 pg, 25-1600 pg, 6.25-200 pg, and 6.25-200 pg, respectively.

The antisera for pregnenolone and progesterone were purchased from Dr. G. E. Abraham and the specifications of these have been published (Abraham et al., 1973, 1971). Antiserum to the estrogens (gift from Dr. B. V. Caldwell) binds at a final dilution of 120,000 approximately 50% of tritiated estradiol-17B. It binds equally well with estrone and has a cross reaction of 10% with estriol. After specific chromatographic separation the antiserum to estrogen can be used for determining levels of both estrone and estradiol-17B (Orczyk et al., 1974).

Assay for gonadotropins. — Plasma LH and FSH were determined by using double antibody radioimmunoassay which was modified according to the procedures described by Gay et al. (1970) and Niswender et al. (1968). The sensitivities of the assays for LH and FSH were 0.5 and 5 ng, respectively, and the intra-assay coefficient of variation was 10%.

Statistics. — The method for contrasting differences between mean values of young and senescent mice was the Student's "t" test.

RESULTS

The number of embryos before and after implantation was significantly lower in senescent females than in young females on days 4, 9, and 16 of pregnancy (Table 1). On day 1 of pregnancy there was no statistical difference in the number of eggs ovulated, although a larger number was abnormal (fragmented or degenerating) in senescent females. There were differences between the two age groups on days 4 and 9 of pregnancy because no embryos or implantation sites were found in a large number of aged females. If these females are excluded from the data, the

Table 1. Effects of Maternal Age on the Numbers of Preimplantation Embryos and Implantation Sites in Young (Y) and Senescent (S) C57BL/6 Mice.

Day of Pregnancy	No. of Females	Mean No. of Corpora Lutea ± SEM	Mean No. of Preimplantation Embryos ± SEM			Mean No. of Implantation Sites ± SEM		
			Normal	Abnormal	Total	Normal	Resorptions	Total
1	Y 20(2)[a]	----------	6.8 ± 0.78	0.5 ± 0.41	7.3 ± 0.71		----------	
	S 18(1)	----------	6.9 ± 0.84	1.2 ± 0.34	8.1 ± 0.78		----------	
4	Y 21(1)	9.1 ± 0.32	6.2 ± 0.64	0.8 ± 0.40	7.0 ± 0.54		----------	
	S 23(8)	6.7 ± 0.85[b]	2.4 ± 0.57[b]	2.0 ± 0.51	4.4 ± 0.86[b]		----------	
9	Y 23(4)	8.5 ± 0.51		---------		6.8 ± 0.79	0.0	6.8 ± 0.79
	S 24(11)	8.4 ± 0.65		---------		3.9 ± 0.78[b]	0.1 ± 0.04	4.0 ± 0.79[b]
16	Y 21	9.6 ± 0.43		---------		7.5 ± 0.43	0.3 ± 0.12	7.8 ± 0.44
	S 20	10.4 ± 0.46		---------		2.8 ± 0.29[c]	3.6 ± 0.34	6.4 ± 0.30[b]

[a]Number of females in which no embryos or implantation sites were found.
[b]Significantly different from young animals ($p < 0.02$).
[c]Significantly different from young animals ($p < 0.001$).

number of preimplantation embryos or implantation sites is similar in the two groups (day 4: young 7.3 ± 0.44 vs senescent 6.7 ± 0.82; day 9: young 8.3 ± 0.52 vs senescent 7.3 ± 0.31). However, on day 4, 46% of the embryos from aged females were abnormal. On day 16, only females visibly pregnant were bled, but there was still a significant difference between the numbers of implantation sites in the two groups. This difference became highly significant ($p < 0.001$) because a comparison of the numbers of normal fetuses revealed the presence of a large number of resorptions in senescent females. Every senescent female exhibited normal fetuses (1-6) and resorptions (2-6), whereas only 5 younger females contained any resorptions (1-2). These results agree with data reported by others for senescent mice (Biggers et al., 1962; Harman & Talbert, 1970; Talbert & Krohn, 1966).

On day 4 of pregnancy, the numbers of corpora lutea in young and aged females were significantly different because there were no visible corpora lutea on ovaries of four aged females (Table 1). These females had definitely mated, although the ovaries were atrophied in two of the females and another female had previously mated twice but never delivered young. On days 9 and 16, the numbers of corpora lutea were not statistically different between the two age groups, indicating the majority of the senescent females was still capable of ovulating a normal number of eggs.

The only difference in the peripheral levels of hormones assayed during the first day of pregnancy was a significantly lower concentration of estradiol-17B in senescent females (Table 2). On day 4, however, estradiol-17B was significantly elevated ($p < 0.05$) in aged females as was FSH ($p < 0.005$). FSH levels continued to remain significantly higher in senescent animals on days 9 and 16. No other statistical differences occurred in the levels of the other hormones during these stages of pregnancy between the two age groups. There were also no consistent differences in the plasma concentrations of hormones from females with or without preimplantation embryos or implantation sites; therefore, these data were combined in Table 2.

DISCUSSION

Harman and Talbert (1970) examined the ovaries of senescent C57BL/6 mice (12-15 mo of age) on the 8th day of gestation and found recognizable corpora lutea in only 23-38% of the females, and of those females, more than 50% exhibited degenerating corpora lutea. Gosden (1974) also described smaller luteal cells and smaller corpora lutea in aged

Table 2. Circulating Plasma Levels of Various Hormones During Pregnancy in Young (Y) and Senescent (S) C57BL/6 Mice.

Day of Pregnancy		Assays per Hormone: Steroids	Gonadotropins	Mean Hormonal Concentration ± SEM: Pregnenolone (ng)	Progesterone (ng)	Estrone (pg)	Estradiol-17B (pg)	LH (ng)	FSH (ng)
1	Y	7	7	5.9 ± 0.9	6.3 ± 2.3	94.9 ± 9.3	223.3 ± 79.0	19.4 ± 3.8	471.1 ± 106.6
	S	7	7	6.7 ± 1.9	8.9 ± 3.6	71.9 ± 10.9	36.0 ± 2.9[a]	44.4 ± 12.8	665.3 ± 98.8
4	Y	8	7	5.5 ± 1.5	26.3 ± 1.8	89.3 ± 11.4	55.2 ± 7.5	26.2 ± 3.0	137.4 ± 15.2
	S	10	7	9.3 ± 2.0	19.4 ± 2.9	76.7 ± 5.7	179.9 ± 45.1[a]	20.3 ± 3.8	501.2 ± 96.2[c]
9	Y	9	7	9.2 ± 1.8	34.1 ± 3.2	71.1 ± 13.7	69.9 ± 12.5	26.4 ± 4.8	227.7 ± 28.6
	S	10	8	9.9 ± 2.8	26.1 ± 4.5	95.5 ± 10.8	77.3 ± 13.4	21.6 ± 4.4	401.7 ± 49.5[b]
16	Y	10	7	14.3 ± 3.4	70.5 ± 7.4	81.1 ± 10.5	92.0 ± 37.8	19.0 ± 1.9	161.9 ± 16.7
	S	10	8	23.2 ± 4.6	55.4 ± 8.7	84.9 ± 7.4	211.4 ± 70.4	16.7 ± 2.2	451.0 ± 62.0[d]

[a]Significantly different from young animals ($p < 0.05$).
[b]Significantly different from young animals ($p < 0.02$).
[c]Significantly different from young animals ($p < 0.005$).
[d]Significantly different from young animals ($p < 0.001$).

CBA/H-T6 mice on day 4 of pregnancy and Albrecht et al. (1975) described the presence of less Δ^5-3B-hydroxysteroid, determined histochemically, in corpora lutea of aged C57BL/6 mice which had failed to maintain pregnancy. All of these researchers contend that luteal failure is the primary cause for unsuccessful implantation and a decreasing litter size in senescent mice. Talbert (1971), however, in a subsequent study emphasized that the degenerating corpora lutea in older mice could be a result of early embryonic death rather than the cause of embryonic death. The present study did not confirm the large numbers of females having no recognizable corpora lutea, although 4 senescent females on day 4 appeared to have no corpora lutea and 2 senescent females on day 9 only exhibited one corpus luteum. The ovaries of some aged females were distinctly smaller and some did not appear to have normal corpora lutea. None of the ovaries from either age group was histologically examined; therefore, it is not known how many older females contained degenerating corpora lutea.

There was no evidence from the circulating plasma levels of the hormones examined to show a hormonal deficiency in older females, except for lower estradiol-17B levels on day 1 of pregnancy. There were no consistent differences in the concentrations of various hormones in mated aged females with or without preimplantation embryos or implantation sites. Since the mean concentration of progesterone and LH, although not significantly different, was consistently lower in senescent females when compared with young females during days 4, 9, and 16 of pregnancy, it is possible that a larger sample size could result in statistical differences. However, the present data agree with those reported for senescent rabbits in which peripheral plasma progestin levels were not significantly different from young rabbits on day 12 of pregnancy, even though 5 of 9 aged females had no viable fetuses. On day 24, 3 of 4 aged rabbits without viable fetuses had plasma progestins below the detectable limits of the assay employed, indicating the absence of fetuses resulted in luteal regression, rather than the reverse effect (Spilman et al., 1972). In the young mature rat the maintenance of progesterone secretion by the corpus luteum is dependent upon LH levels between 8 and 11 days of pregnancy (Morishige & Rothchild, 1974; Pepe & Rothchild, 1974; Raj & Moudgal, 1970). If this dependency is similar in the mouse, the corpora lutea of the aged female could be adequately functioning, since there were no significant differences in the concentrations of progesterone or LH of the two age groups on day 9. There was, however, an

obvious gonadotropin imbalance in aged mice on days 4, 9, and 16 of pregnancy because FSH was 2-4 times higher than the level found in younger females. This may be an indication of a dysfunctioning hypothalamic-pituitary complex in the senescent mouse, since the concentrations of FSH in pituitary glands is 2-3 times greater in older rats (Labhsetwar, 1969). It had been suggested from previous studies that a hormonal imbalance or deficiency exists in senescent hamsters and C57BL/6 mice because of the retention of blastocysts during pregnancy and the presence of eggs from previous estrous cycles within oviducts (Parkening, 1976), and because of a delay in the fertilization of eggs from senescent hamsters (Parkening & Soderwall, 1975). It is not known if a delay in fertilization exists in eggs of senescent C57BL/6 mice, but differences in estradiol-17B levels of aged females on days 1 and 4 of pregnancy could disrupt the synergistic effect of progesterone and estrogen on muscular activity which is responsible for the normal passage of eggs through the oviduct.

The uterus of aged animals appears to be responsible for considerable postimplantation loss in those females with implantation sites. This was primarily evident on day 16 of pregnancy when every aged female had 1 to 6 resorptions. Since the concentration of circulating hormones was not significantly different between young and senescent females, except for considerably higher FSH levels, this may be an indication of vascular impairment of the aged uterus. Four of the aged females contained 1-2 fetuses that appeared normal, but were markedly smaller than other fetuses on day 16 of pregnancy. A reduction in the rate of uterine blood flow has been suggested as a cause for reduced litter sizes in senescent rabbits (Larson & Foote, 1972) and hamsters (Parkening & Soderwall, 1974). The administration of exogenous progesterone to aged CBA/H-T6 mice and daily supplementation with prolactin from 1 to 18 days of pregnancy in aged C57BL mice failed to improve the number of implantation sites (Gosden, 1975), also suggesting the uterus may be less capable of responding to hormones. This may result from the inability of the aged uterus to bind hormones, from an impairment of circulation or a combination of both factors.

This study indicates that the failure of the corpus luteum to maintain itself in the senescent mouse ovary may not be due to the lack of circulating gonadotropins but to some intrinsic properties of the aging ovary. While it appears that aging adversely affects ovulation in some older mice, in those ovulating, the circulating levels of the hormones assayed suggested that the corpora lutea were still functional in most females, although it is not known what local systemic differences occur in hormonal levels. It does appear that preimplantation loss is an important factor in the reduction of litter size during aging in this strain of mouse and that the aged uterus is almost certainly responsible for some postimplantation loss.

SUMMARY

Young (3-5 mo of age) and senescent (12-15 mo of age) multiparous C57BL/6 mice were mated with young males (3-6 mo of age) and the numbers of preimplantation embryos and implantation sites determined on days 1 (day of plug), 4, 9, and 16 of pregnancy. The numbers of viable embryos were significantly lower ($p < 0.02$ to $p < 0.001$) in senescent females compared with young females on all days except day 1 of pregnancy. Blood plasma collected from the females of both age groups was analyzed by radio immunoassay to determine circulating levels of pregnenolone, progesterone, estrone, estradiol-17B, LH, and FSH. Estradiol-17B was significantly lower ($p < 0.05$) on day 1 and higher ($p < 0.05$) on day 4 in older females, whereas FSH was consistently higher on days 4, 9, and 16 ($p < 0.02$ to $p < 0.001$) in senescent females when comparing concentrations with young females. Failure of the corpora lutea to maintain themselves did not appear to be the cause of embryonic loss in senescent mice, as reflected by the circulating level of hormones. While the ovary may contribute to some embryonic loss in aged females, primarily through the inability of ova to ovulate, the declining litter size in aged mice appears to be largely the result of abnormal embryos and the inability of the uterus to maintain implanted embryos.

REFERENCES

Abraham, G. E., Buster, J. E., Kyle, F. W., Corrales, P. C. & Teller, R. C. Radioimmunoassay of plasma

pregnenolone. *Journal of Clinical Endocrinology & Metabolism*, 1973, *37*, 40-45.
Abraham, G. E., Swerdloff, R., Tulchinsky, D. & Odell, W. D. Radioimmunoassay of plasma progesterone. *Journal of Clinical Endocrinology & Metabolism*, 1971, *32*, 619-624.
Albrecht, E. D., Koos, R. D., & Wehrenberg, W. B. Ovarian Δ^5-3B-hydroxysteroid dehydrogenase and cholesterol in the aged mouse during pregnancy. *Biology of Reproduction*, 1975, *13*, 158-162.
Biggers, J. D. Problems concerning the uterine causes of embryonic death, with special reference to the effects of aging of the uterus. *Journal of Reproduction & Fertility Suppl.*, 1969, *8*, 27-43.
Biggers, J. D., Finn, C. A., & McLaren, A. Long term reproductive performance of female mice II. Variation of litter size with parity. *Journal of Reproduction & Fertility*, 1962, *3*, 313-330.
Blaha, G. C., & Leavitt, W. W. Ovarian steroid dehydrogenase histochemistry and circulating progesterone in aged golden hamsters during the estrous cycle and pregnancy. *Biology of Reproduction*, 1974, *11*, 153-161.
Finn, C. A. The ageing uterus and its influence on reproductive capacity. *Journal of Reproduction & Fertility Suppl.*, 1970, *12*, 31-38.
Gay, V. L., Niswender, G. D., Midgley, A. R., Jr., & Reichert, L. E., Jr. Radioimmunoassay for rat FSH with antiserum to human FSH and radioiodinated rat FSH. *Federation Proceedings*, 1970, *29*, 439 (abstract).
Gosden, R. G. Corpus luteum adequacy in the ageing pregnant mouse. *European Journal of Obstetrics & Gynecology & Reproductive Biology Suppl.*, 1974, *4*, 109-111.
Gosden, R. G. Ovarian support of pregnancy in aging inbred mice. *Journal of Reproduction & Fertility*, 1975, *42*, 423-430.
Harman, S. M., & Talbert, G. B. The effect of maternal age on ovulation, corpora lutea of pregnancy, and implantation failure in mice. *Journal of Reproduction & Fertility*, 1970, *23*, 33-39.
Howland, B. E., & Preiss, C. Effects of aging on basal levels of serum gonadotropins, ovarian compensatory hypertrophy and hypersecretion of gonadotropins after ovariectomy in female rats. *Fertility & Sterility*, 1975, *26*, 271-276.
Labhsetwar, A. P. Age-dependent changes in the pituitary — gonadal relationship. II. A study of pituitary FSH and LH content in the female rat. *Journal of Reproduction & Fertility*, 1969, *20*, 21-28.
Labhsetwar, A. P. Ageing changes in pituitary-ovarian relationships. *Journal of Reproduction & Fertility Suppl.*, 1970, *12*, 99-117.
Larson, L. L., & Foote, R. H. Uterine blood flow in young and aged rabbits. *Proceedings of Society for Experimental Biology & Medicine*, 1972, *141*, 67-69.
Larson, L. L., Spilman, C. H., & Foote, R. H. Uterine uptake of progesterone and estradiol in young and aged rabbits. *Proceedings of Society for Experimental Biology & Medicine*, 1972, *141*, 463-466.
Leathem, J. H., & Shapiro, B. H. Aging and ovarian Δ^5-3B-hydroxysteroid dehydrogenase in rats. *Proceedings of Society for Experimental Biology & Medicine*, 1975, *148*, 793-794.
Maibenco, H. C., & Krehbiel, R. H. Reproductive decline in aged female rats. *Journal of Reproduction & Fertility*, 1973, *32*, 121-123.
Morishige, W. K. & Rothchild, I. Temporal aspects of the regulation of corpus luteum function by luteinizing hormone, prolactin and placental luteotrophin during the first half of pregnancy in the rat. *Endocrinology*, 1974, *95*, 260-274.
Niswender, G. D., Midgley, A. R., Jr., Monroe, S. E., & Riechert, L. E., Jr. Radioimmunoassay for rat luteinizing hormone with antiovine LH serum and ovine LH-^{131}I. *Proceedings of Society for Experimental Biology & Medicine*, 1968, *128*, 807-811.
Orczyk, G. P., Caldwell, B. V., & Behrman, H. R. In B. M. Jaffe & H. R. Behrman Eds., Estrogens: Estradiol, estrone and estriol. In *Methods of hormone radioimmunoassay*. Academic Press, New York, 1974.
Parkening, T. A. Retention of ova in oviducts of senescent mice and hamsters. *Journal of Experimental Zoology*, 1976, *196*, 307-314.
Parkening, T. A., & Soderwall, A. L. Preimplantation stages from young and senescent golden hamsters: Presence of succinic dehydrogenase and non-viable ova. *Journal of Reproduction & Fertility*, 1973, *35*, 373-376.
Parkening, T. A., & Soderwall, A. L. Histochemical localization of glycogen in preimplantation and implantation stages of young and senescent golden hamsters. *Journal of Reproduction & Fertility*, 1974, *41*, 285-295.
Parkening, T. A., & Soderwall, A. L. Delayed fertilization and preimplantation loss in senescent golden hamsters. *Biology of Reproduction*, 1975, *12*, 618-631.
Peng, M. T., & Peng, Y. M. Changes in the uptake of tritiated estradiol in the hypothalamus and adenohypophysis of old female rats. *Fertility & Sterility*, 1973, *24*, 534-539.
Pepe, G. J., & Rothchild, I. A comparative study of serum progesterone levels in pregnancy and in various types of pseudopregnancy in the rat. *Endocrinology*, 1974, *95*, 275-279.
Purvis, K., Brenner, P. F., Landgren, B. M., Cekan, Z., & Diczfalusy, E. Indices of gonadal function in the human male. I. Plasma levels of unconjugated steroids and gonadotrophins under normal and pathological conditions. *Clinical Endocrinology*, 1975, *4*, 237-246.
Raj, H. G. M., & Moudgal, N. R. Hormonal control of gestation in the intact rat. *Endocrinology*, 1970, *86*, 874-889.
Saksena, S. K., Lau, I. F., & Chang, M. C. The inhibition of the conversion of testosterone into 5α-dihydrotestosterone in the reproductive organs of the male rat. *Steroids*, 1976, *27*, 751-757.
Shapiro, M., & Talbert, G. B. The effect of maternal age on decidualization in the mouse. *Journal of Gerontology*, 1974, *29*, 145-148.
Spilman, C. H., Larson, L. L., Concannon, P. W., & Foote, R. H. Ovarian function during pregnancy in young and aged rabbits: Temporal relationship between fetal death and corpus luteum regression. *Biology of Reproduction*, 1972, *7*, 223-230.
Talbert, G. B. Effect of maternal age on postimplantation reproductive failure in mice. *Journal of Reproduction & Fertility*, 1971, *24*, 449-452.
Talbert, G. B., & Krohn, P. L. Effect of maternal age on viability of ova and uterine support of pregnancy in mice. *Journal of Reproduction & Fertility*, 1966, *11*, 399-406.
Thorneycroft, I. H., & Soderwall, A. L. Ovarian morphological and functional changes in reproductively senescent hamsters. *Anatomical Record*, 1969, *165*, 349-354.
Watkins, B. E., Meites, J., & Riegle, G. D. Age-related changes in pituitary responsiveness to LHRH in the female rat. *Endocrinology*, 1975, *97*, 543-548.