BIOLOGY OF REPRODUCTION 18, 658–662 (1978)

Hormone Patterns in Pregnant or Pseudopregnant Hamsters After Unilateral Ovariectomy or Hysterectomy

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ABSTRACT

Hormone levels were compared at 1000 h from Days 2–12 between pregnant (PREG) and pseudopregnant (PSP) hamsters. Serum progesterone (P) fell drastically in the PSP animals between Days 6 and 8 indicating that placental luteotropic function is normally established during this period. In hysterectomized-pseudopregnant hamsters (HS-PSP), serum P was maintained past Day 6 at approximately 8 ng/ml—the level found in intact PSP animals until Day 6. Hamster unilaterally ovariectomized on Day 1 of pregnancy (ULO-PREG), despite having only one half the normal number of corpora lutea (CL), had serum P levels between Days 2 and 12 which did not differ significantly from the intact-PREG group. There was also no increase in mean luteal weight in the ULO-PREG animals.

The nonluteal weight (total ovarian weight minus total luteal weight) of the remaining ovary in the ULO-PREG animals was significantly increased over the PREG group, largely representing compensatory increase in the number of antral follicles. However, serum levels of FSH, LH and PRL did not differ between Days 4 and 10 for the 2 groups. The HS-PSP animals had significantly higher levels of PRL and LH on Days 8–12 compared to values in the intact-PREG group. Despite having reduced nonluteal weight and presumably fewer antral follicles, the HS-PSP animals on Day 12 had as much serum estradiol (E_2) as the ULO or intact-PREG hamsters. Hence, the number of antral follicles does not necessarily correlate with peripheral levels of E_2.

INTRODUCTION

In the pregnant hamster, the definitive placenta is formed (Ward, 1948) and probably begins producing luteotropic hormone(s) about Day 8 of the 16 day gestation (Greenwald, 1967). A previous investigation from this laboratory (Greenwald, 1974) reported that the ensuing pseudopregnancy after hysterectomy on Day 1 of pregnancy was prolonged to approximately 16 days; however, the peripheral level of progesterone at Day 12 in these hysterectomized-pseudopregnant (HS-PSP) animals was only half the value at Day 12 of normal pregnancy. Furthermore, the number of antral follicles in the HS-PSP hamsters was one-sixth the normal number in pregnant (PREG) animals, even though the pituitary levels of both FSH and LH between the 2 groups were not statistically different. The pituitary data, obtained by bioassays, suggested that the prolonged pseudopregnancy depended on the sustained release of prolactin (PRL) and gonadotropins but probably at lower levels than in the pregnant animal.

Accepted November 3, 1977.
Received August 31, 1977.

The principal objective of the present study was to compare peripheral concentrations of estradiol (E_2), progesterone (P), FSH, LH and PRL in pregnant hamsters with the levels in intact pseudopregnant (INT-PSP) and HS–PSP hamsters to determine when the hormonal profiles diverge, thus providing additional insight on the time of establishment of placental function.

Following unilateral ovariectomy (ULO) of the pregnant hamster, compensatory hypertrophy of the remaining ovary occurs, consisting primarily of an increase in the number of antral follicles (Chatterjee and Greenwald, 1971). Because of these alterations in follicular development after ULO, a second objective was to compare hormone levels in pregnant hamsters subjected to ULO versus intact pregnant animals.

MATERIALS AND METHODS

Golden hamsters (*Mesocricetus auratus*) were mated after 3 consecutive 4 day cycles and the presence of sperm in the vaginal smear the next morning designated Day 1 of pregnancy. The animals were maintained on a 14 h light: 10 h dark schedule with lights on at 0500 h (CST). At 0900 h of Day 1 of pregnancy the sperm-positive animals were separated into 4 groups and treated as follows:

Group 1 Intact pregnant animals (PREG). In some instances, the hamsters were anesthetized with ether.

Group 2 Unilaterally ovariectomized pregnant anmals (ULO-PREG). ULO was performed under ether anesthesia; alternate left and right ovaries were removed.

Group 3 Intact pseudopregnant hamsters (INT-PSP). Oviducts were severed at the utero-tubal junction thereby preventing passage of the embryos into the uterine horns with subsequent absence of implantation.

Group 4 Hysterectomized-pseudopregnant hamsters (HS-PSP). Pseudopregnancy was induced by complete resection of the uterine horns; nembutal anesthesia was used (as in Group 3) due to the lengthier surgical procedure.

Animals were then decapitated between 0900 h and 1000 h at 2-day intervals between Days 2 and 12 (5 hamsters/day/group) and serum was saved for radioimmunoassays (RIA). The pairs of ovaries were weighed (except for Group 2). One ovary was weighed and after all corpora lutea (CL) had been dissected and weighed as a pool, the remaining nonluteal ovary was reweighed.

Determination of FSH, LH or PRL In Sera by Radioimmunoassay (RIA)

Double antibody, rat:rat RIAs for FSH and PRL were used and have been described previously (Bast and Greenwald, 1974). An ovine:ovine LH-RIA system (Niswender et al., 1971) was used and the general methodology for the LH assay was the same as the FSH and PRL assays. Anti-ovine LH serum (GDN-15) and ovine LH for iodination (LER-1056-C2) were kindly provided by Drs. G. D. Niswender and L. E. Reichert, Jr., respectively. Hormone concentrations are expressed in terms of NIAMDD-rat FSH-RP-1 (potency estimated by NIAMDD was 2.1 × NIH-FSH-S1), NIAMDD-rat LH-RP-1 (0.03 × NIH-LH-S1) or NIAMDD-rat prolactin-RP-1 (11IU/mg).

Determination of Estradiol-17β (E_2) and Progesterone (P) in Sera by Radioimmunoassay (RIA)

A highly specific antiserum to estradiol-17β-6-(O-carboxymethyl) oxime-bovine serum albumin was generously provided by Dr. D. Exley and associates; the antibody has been characterized (Exley et al., 1971). Anti-progesterone serum (IHT-R-11-1516-1) prepared against 11α-succinyl-progesterone-bovine serum albumin was supplied by Dr. I. H. Thorneycroft which also has been characterized (Thorneycroft and Stone, 1972).

The methodologies for the RIAs were similar to the procedures described in detail by Hotchkiss et al. (1971) for an estrogen RIA. Samples of sera (300 μl in the E_2-RIA; 10 μl in the P RIA) were diluted to 1 ml with distilled water and then extracted once with 5 ml of fresh anhydrous diethyl ether; the extract was evaporated and used without additional purification. The reference steroids for the standard curves were added to 12 × 75 mm culture tubes, which had been pretreated with a 5 ml ether extract of 1 ml distilled water. The radioactive steroids used in the respective RIA were (2,4,6,7-^{3}H)-estradiol-17β and (1,2,6,7-^{3}H)-progesterone (SA = 105 and 81.1 mCi/mmol, respectively; New England Nuclear, Boston, MA).

Determination of steroid content was calculated from the transformed standard curves (logit-response vs log-dose) after linear regression analysis and included correction for procedural loss. The average recovery percentages (±SE) for labeled E_2 and P in the extraction procedure were 80.9 ± 2.4% and 72.9 ± 0.8% (n = 76), respectively. The lower limits of sensitivity in these assays were 2.0 pg E_2 and 4.1 pg P. When 12 pg or 37 pg of unlabeled P were added to serum from ovariectomized hamsters and then carried through the P RIA procedures, 15.7 ± 1.9 pg and 32.1 ± 4.4 pg P, respectively, were the recovered net yields. In the E_2 RIA, 18.5 ± 0.2 pg and 63.4 ± 2.9 pg E were the respeotive yields when 16.6 pg and 66.2 pg of unlabeled E were added. Serum (300 μl) from ovariectomized-hypophysectomized hamsters contained 12 pg E, while 10 μl of the serum had 6 pg P.

Statistical significance was determined at the 0.05 probability level and was assessed by Student's t test (Snedecor and Cochran, 1967).

RESULTS

Ovarian Weights of Pregnant and PSP Hamsters (Fig. 1)

Paired ovarian weights were similar through Day 8 of pregnancy or PSP. Thereafter there was a sharp drop in the weights of the INT-PSP group contrary to the steady state maintained by the HS-PSP animals and the abrupt increase in ovarian weight of the pregnant animals. For the INT-PSP group, the decline in ovarian weight after Day 8 was paralleled by a concomitant decline in luteal and nonluteal weights. ULO-PREG animals had mean luteal weights equal to the intact pregnant group, but for the former group, the nonluteal ovarian weight was significantly elevated by Day 8.

Steroid Profiles in Pregnant and Pseudopregnant (PSP) Hamsters (Fig. 2)

There was no difference in serum progesterone between PREG and ULO-PREG groups. For the INT-PSP animals, serum P dropped significantly by Day 8, although luteal weights were still high. Serum P was sustained past Day 6 at about 8 ng/ml in the HS-PSP animals; however, for this group, P levels differed significantly on Days 10 and 12 from the pregnant animals.

Serum levels of E_2 were comparable between the 2 pregnant groups at all time inter-

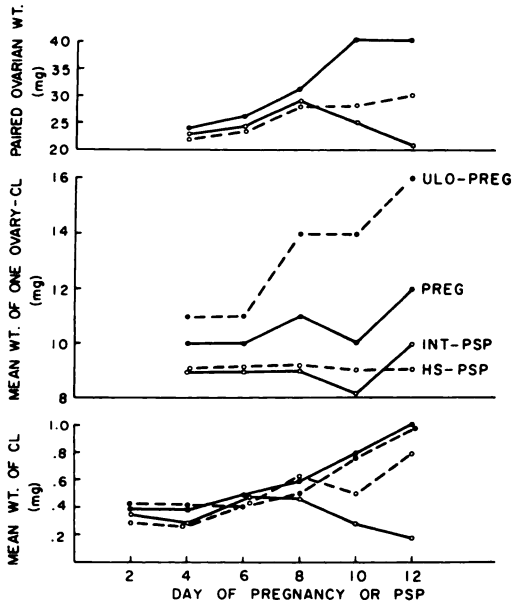

FIG. 1 Comparisons of weights of ovarian tissues in pregnant or pseudopregnant hamsters. PREG = intact pregnant; ULO-PREG = unilaterally ovariectomized pregnant; INT-PSP = intact pseudopregnant (uterotubal junction ligated); HS-PSP = hysterectomized and pseudopregnant. All operations were performed on Day 1 of pregnancy, the first day of a sperm-positive vaginal lavage after mating. S.E.s of the means are not shown in the figure because they were small and encompassed by the symbols for the means.

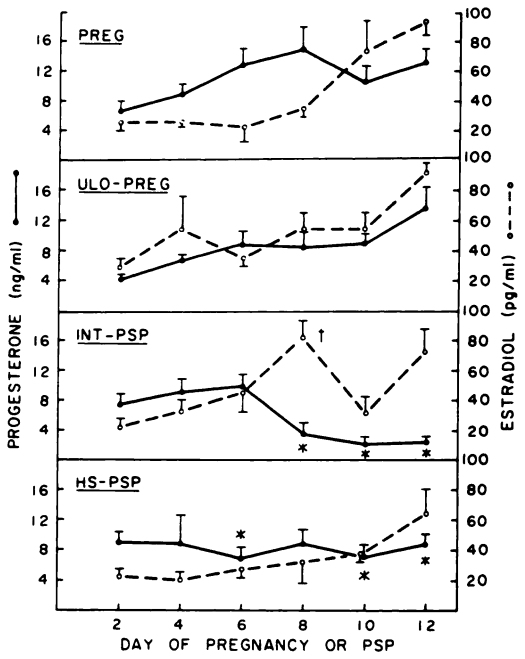

FIG. 2. The peripheral concentrations of progesterone and estradiol-17β in pregnant or pseudopregnant hamsters (abbreviations are the same as in Fig. 1). The asterisk above or the cross beside a symbol indicates that the particular level of hormone represented by the symbol was significantly different from the respective level in the intact, pregnant animal.

vals, but increased significantly at Day 8 in the INT-PSP hamsters.

FSH, LH, and PRL in Pregnant and PSP Hamsters (Fig. 3)

For the pregnant hamsters, serum PRL peaked on Days 6 and 8, followed by a steady fall to Day 12; this correlated with a trend for higher levels of serum FSH on Days 10 and 12. Serum FSH and LH did not differ from the values in pregnant animals except on Day 12 when serum LH and PRL were significantly higher in the ULO-PREG group.

The INT-PSP animals on Day 8 showed significant differences in LH and FSH from the pregnant groups. On Day 12, serum FSH was significantly reduced in the INT-PSP group. HS-PSP animals showed sustained serum levels of PRL and LH on Days 8–12, whereas there was a gradual decline in these hormones in the pregnant hamsters.

DISCUSSION

After semispaying on the first day of pregnancy, the remaining ovary shows a marked increase in nonluteal weight (Fig. 1) providing further evidence for a compensatory increase in the number of antral follicles. However, the increase in follicular development is not associated with any change in serum FSH or LH, at least not at 0900 h to 1000 h. This caveat is implicit throughout this discussion; i.e., the possibility cannot be discounted that significant hormonal changes might occur at times other than the one interval sampled.

ULO on Day 1 of pregnancy obviously causes an approximate halving in the number of CL and yet serum progesterone levels are the same for the intact and ULO pregnant groups (Fig. 2), unassociated with any increase in luteal weight in the ULO animals (Fig. 1). The maintenance of normal peripheral levels of P after ULO presumably results from an

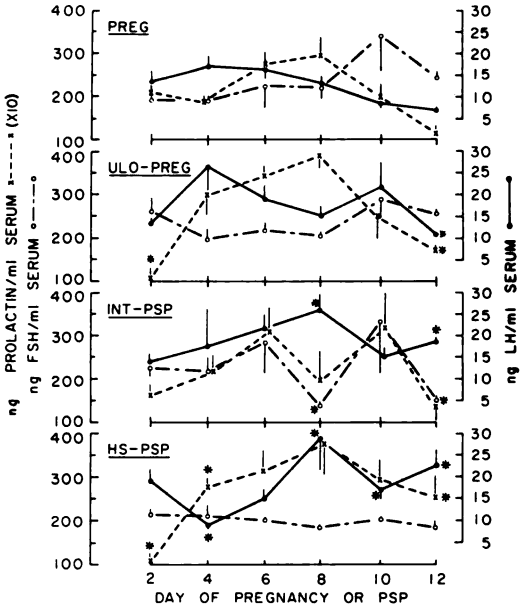

FIG. 3 Levels of gonadotropins and prolactin in the serum of pregnant or pseudopregnant hamsters (abbreviations are the same as in Fig. 1). The asterisk above or beside a symbol indicates that the particular level of hormone represented by the symbol was significantly different from the respective level in the intact, pregnant animal.

increased secretion rate of the hormone but alternatively, an altered metabolic clearance rate could also account for the maintenance of serum levels of P. At this point we cannot distinguish between these two possibilities, although in the rat, the metabolic clearance rate is a very stable phenomenon, unaffected by ovariectomy or other experimental manipulations (Pepe and Rothchild, 1973).

The stability of serum levels of P in the face of ULO in the hamster contrasts with the situation in the rat where semispaying causes a drop in serum P correlated with the number of CL left *in situ* (Elbaum et al., 1975).

Comparison of P levels of INT-PSP and pregnant hamsters indicates that placental function is established between 6 and 8 days (Fig. 2). The duration of pseudopregnancy in the hamster as measured by the onset of ovulation is 10.0 ± 0.3 days (Greenwald, 1974) which is consistent with the present findings. The decline in P in the PSP hamsters between Days 6 and 8 is associated with a decline in PRL and FSH and a significant increase in serum E_2. The E_2 level on Day 8 of PSP is comparable to that found on Day 3 of the cycle (Baranczuk and Greenwald, 1973) indicating that ovulation is imminent.

The values for serum P abruptly fall between Days 6 and 8 in the INT-PSP group, whereas P is sustained at about 8 ng/ml in the HS-PSP group at least through Day 12. The maintenance of P at normal pseudopregnant values results from sustained pituitary activity, especially for PRL. In contrast, in the pregnant hamsters, serum PRL declines after Day 8 presumably as a placental source of a prolactin-like hormone is established. Serum P is significantly elevated in the pregnant hamsters at Days 10 and 12 compared to the HS-PSP group. For the latter group, could the elevated levels of LH on Days 10 and 12 be exerting some luteolytic activity?

Contrary to our previous expectations based on pituitary concentrations of FSH and LH (Greenwald, 1974), the present study reveals that circulating levels of gonadotropins and PRL in HS-PSP hamsters are not lower than in the pregnant animal (Fig. 3).

Pregnant or HS-PSP hamsters injected on Day 12 with 20 IU HCG ovulate 30.6 and 5.6 ova, respectively (Greenwald, 1974). On day 12 of prolonged PSP, the nonluteal ovary weighs considerably less than its counterpart in the pregnant hamster (Fig. 1). Collectively, these results suggest that on Day 12 there are considerably fewer antral follicles in the HS-PSP group than in the pregnant group but despite a probable 5-fold difference in numbers of mature antral follicles, serum E_2 levels at Day 12 do not differ significantly between the 2 groups (Fig. 2). Hence, numbers of antral follicles may not necessarily correlate with peripheral levels of E_2. Furthermore, the difference in numbers of antral follicles between the pregnant and HS-PSP groups on Day 12 cannot be explained by differences in FSH levels. Perhaps the higher circulating levels of PRL or LH in the HS-PSP group are responsible for the depression in follicular activity. Experiments are in progress to test this possibility.

ACKNOWLEDGMENTS

The research was supported by grants from NIH (HD00596) and the Ford Foundation. We thank NIAMDD for providing the rat FSH and PRL preparations used in the RIAs.

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