Effects of superovulation on fetal development and hormone levels in the pregnant hamster

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Summary. Treatment of hamsters with 30 i.u. PMSG induced ovulation of 46 ova/animal, 90% of which were fertilized. CL growth was normal. About 27 zygotes/animal implanted and at term (Day 16) the litter size of the PMSG-treated hamsters was 21 compared to the normal number of 10. Parturition occurred at the normal time. Average weight at birth was about 500 mg less for young of PMSG-treated mothers than for controls.

Differences were found in the serum progesterone and oestradiol concentrations of the treated and control hamsters, but serum FSH and LH levels were similar.

Introduction

In the hamster, injection of 30 i.u. PMSG on Day 1 of the cycle (metoestrus), results 4 days later in the ovulation of 40–50 eggs (Greenwald, 1974). In the present study the effects of PMSG treatment on the course of pregnancy and hormone levels are considered.

Materials and Methods

Adult golden hamsters (*Mesocricetus auratus*) were injected s.c. at 09.00 hours on Day 1 of the cycle (morning of ovulation) with 30 i.u. PMSG (NIAMDD preparation no. 1) in 0.1 ml 0.85% NaCl. The females were caged with males on the evening of Day 4 (oestrus) and the presence of spermatozoa in the vagina the next morning indicated Day 1 of pregnancy. At the times indicated in Table 1, groups of animals were examined. Sufficient blood for radioimmunoassay of various hormones was withdrawn by cardiac puncture from unanaesthetized animals which were then killed by an overdose of ether. The ovaries were removed and weighed; 8 CL were then dissected from one ovary with watchmaker forceps and weighed collectively to determine the mean luteal weight.

As a control for the PMSG treatment, groups of normal pregnant hamsters were killed on Days 1, 2, 8, 14, 15 and 16 and hormone levels and other endpoints were compared.

Assays. The ovine:ovine LH assay was based on the procedure of Niswender, Reichert, Midgley & Nalbandov (1971) with the modifications for the hamster described by Bast & Greenwald (1974a). The rat:rat FSH assay, as modified for the hamster by Bast & Greenwald (1974b), was used with NIAMDD-anti-rat FSH-serum 6. The results are expressed in terms of NIAMDD-rat-LH-RP1 and NIAMDD-rat-FSH-RP1.

Serum levels of oestradiol-17 β were determined by radioimmunoassay using a specific antibody provided by Dr D. Exley. The antibody was produced with hapten 17 β -oestradiol-6-(O-carboxymethyl) oxime-bovine serum albumin. Cross-reactivity with oestrone was <1%. The assay procedure was based on the description of Exley, Johnson & Dean (1971) as modified by McCormack & Greenwald (1974). In the present study, the lower limit of sensitivity of the standard curve was 2 pg. The recovery of [2,4,6,7-³H]oestradiol-17 β standard was 72 $\pm$ 3%. Water blanks yielded undetectable values of oestradiol-17 β . Serum from ovariectomized hamsters, used to determine nonspecific binding effects, gave a mean $\pm$ S.E.M. value of 14 $\pm$ 0.8 pg/ml which was not subtracted from the determinations.

Serum levels of progesterone were determined by radioimmunoassay with a specific antibody (Pool AB 12) produced against 11 α -hydroxyprogesterone-bovine serum albumin and kindly provided by Dr Ali H. Surve. The assay was based on the procedure of Thorneycroft & Stone (1972) as modified for the hamster by Baranczuk & Greenwald (1974). Serum samples of 20 μ l were assayed in duplicate. The lower limit of sensitivity of the standard curve was 8 pg. The recovery of the internal standard ([1,2,6,7- 3 H]progesterone) was $75 \pm 4\%$. Water blanks never exceeded 10 pg. Serum from ovariectomized hamsters contained <1 ng progesterone/ml.

Statistical analysis. Comparisons between mean concentrations of hormones were made with Student's *t* test, with statistical significance determined at the 0.05 probability level.

Results

Effects on fetal numbers and development

During the first 3 days of pregnancy, a mean $\pm$ S.E.M. of 45.5 ± 3.6 ova per hamster was recovered from the oviducts, thus providing a minimal estimate of the number of CL formed (Table 1). On Days 2 and 3, the percentage of fertilized ova was 91 and 87%, respectively, as indicated by the presence of 2–4-cell stages on Day 2 and 4–8-cell stages on Day 3. By 21.00 hours on Day 3, all eggs had migrated into the uterus and most of the ova were transformed into morulae, although a few early blastocysts were also present. In this group there was a discrepancy of approximately 40% between the number of CL (43.7 ± 2.6 /hamster) and ova (Table 1); 90% of the recovered ova were fertilized.

Table 1. Mean ($\pm$ S.E.M.) number of ova, swellings or fetuses and ovarian and luteal weight in hamsters (no. in parentheses) at different days of pregnancy after treatment with 30 i.u. PMSG on Day 1 of the cycle

Day of pregnancy (at 09.00 hours)	No.	PMSG-treated		Control	
		Ovarian wt (mg)	Mean luteal wt (mg)	Ovarian wt (mg)	Mean luteal wt (mg)
	<i>Ova</i>				
1	48.8 ± 4.5 (4)	48.8 ± 4.5	0.30 ± 0.01	—	—
2	54.8 ± 5.5 (4)	50.3 ± 2.4	0.31 ± 0.01	31.9 ± 2.3	0.33 ± 0.01
3	38.5 ± 1.9 (4)	45.6 ± 4.0	0.35 ± 0.01	—	—
3	28.0 ± 0.6 (3)	—	—	—	—
(at 21.00 hours)					
	<i>Swellings</i>				
4	Nidation	45.8 ± 3.8	0.44 ± 0.03	—	—
6	24.5 ± 1.9 (4)	53.0 ± 2.1	0.58 ± 0.05	—	—
8	29.3 ± 1.9 (4)	60.9 ± 3.9	0.72 ± 0.05	32.4 ± 2.4	0.76 ± 0.04
10	28.3 ± 1.6 (4)	83.5 ± 5.3	1.06 ± 0.08	—	—
	<i>Fetuses</i>				
12	22.3 ± 2.4 (4)	85.0 ± 9.0	1.00 ± 0.10	—	—
14	20.8 ± 2.2 (6)	96.0 ± 5.6	1.37 ± 0.07	48.6 ± 1.6	1.50 ± 0.02
15	18.4 ± 3.8 (6)	96.0 ± 4.8	1.46 ± 0.11	51.0 ± 3.6	1.42 ± 0.05
16	20.8 ± 1.5 (6)	79.2 ± 2.3	1.1 ± 0.07	43.0 ± 3.8	1.22 ± 0.07

The number of embryonic swellings/hamster between Days 6 and 10 of pregnancy was 27.3 ± 1.1 , and thereafter fell to 20.4 ± 1.3 over the rest of gestation. On the morning of Day 16 (before parturition) there were approximately twice as many fetuses in the PMMSG-treated hamsters (Table 1) as in the controls (9.7 ± 0.71). A group of 6 PMMSG-treated hamsters had the uterus severed on one side at the uterotubal junction on Day 1 of pregnancy and were therefore unilaterally pregnant on Day 16. They had the same number of fetuses (9.8 ± 1.7) as the intact controls, indicating that the carrying capacity of the uterus was limited to 10 fetuses/horn.

Parturition began in the PMSG-treated hamsters at the same time as in the controls: by 09.00 hours of Day 16. The young born to the PMSG-treated mothers were smaller (1650 ± 50 mg, $N = 75$) and more feeble than those of the controls (2142 ± 46 mg, $N = 32$). By the next morning, 5 of 6 PMSG-treated mothers had eaten their young.

Effects on ovarian and CL weight

Superovulation resulting from PMSG treatment was reflected in a highly significant increase in ovarian weight, evident as early as Day 1 of pregnancy (Table 1). There were no histological differences between the CL of the treated and control animals and the growth of individual CL occurred at the same rate. The ovaries of the PMSG-treated hamsters were therefore heavier because of the greater number of CL.

Effects on hormone levels

These are shown in Table 2. Progesterone concentrations were 2–4 times higher in the treated animals than in the controls, the peak for both being reached on Day 14. Peak levels of oestradiol-17 β also occurred on Day 14, but the differences were significant on Day 8 of pregnancy. The decline of oestradiol on Days 15 and 16 also occurred at a significantly slower rate in control than in treated hamsters.

Table 2. Mean $\pm$ S.E.M. serum concentrations of hormones in PMSG-treated and control (values in parentheses) hamsters (4/group)

Day of pregnancy	Progesterone (ng/ml)	Oestradiol (pg/ml)	LH (ng/ml)	FSH (ng/ml)
1	3.9 ± 1.5	35 ± 1.7	125 ± 9 (30 ± 8)	249 ± 29
2	17.2 ± 3.2 (3.2 ± 1.5)	34 ± 2.5 (26 ± 4.2)	48 ± 6 (38 ± 15)	217 ± 25 (204 ± 35)
3	15.6 ± 1.2	35 ± 4.2	33 ± 8	195 ± 2
4	17.9 ± 3.5	36 ± 3.4	15 ± 2	217 ± 19
6	18.4 ± 6.9	41 ± 1.6	17 ± 6	249 ± 30
8	22.8 ± 2.1 (5.5 ± 1.4)	59 ± 4.8 (90 ± 7.5)	18 ± 6 (18 ± 2)	161 ± 34 (234 ± 11)
10	23.9 ± 2.3	89 ± 8.4	14 ± 4	217 ± 29
12	28.0 ± 4.3	113 ± 10.6	18 ± 1	104 ± 10
14	41.4 ± 4.0 (18.5 ± 2.0)	133 ± 20.3 (138 ± 11)	9 ± 1	237 ± 31 (231 ± 22)
15	31.0 ± 4.5 (19.4 ± 4.9)	49 ± 4.2 (94 ± 14.9)	9 ± 1 (11 ± 1)	183 ± 6 (218 ± 9)
16	< 1 (< 1)	21 ± 1.7 (54 ± 10.6)	17 ± 1	238 ± 18 (243 ± 18)

Peripheral levels of LH and FSH (Table 2) were similar from Day 2 of pregnancy and thereafter. The very high LH value encountered on Day 1 of pregnancy in the PMSG-treated hamsters probably represented cross-reactivity of the ovine:ovine LH assay system with the PMSG given 4 days previously (Ely & Chen, 1968).

Discussion

The present study reveals that in hamsters the extra follicles induced to ovulate by PMSG are physiologically normal as attested by the high percentage of fertilized eggs and the fact that the CL show the normal growth pattern of CL of pregnancy. The litter size at term was increased to double that of control animals.

Considerable species differences exist in the number of fetuses maturing until term after PMSG treatment. In the adult mouse a mean number of 18.9 swellings at 7½ days of pregnancy was reduced

to 10·2 near term, a number not very different from the average of 8·9 living fetuses encountered in control animals (McLaren & Michie, 1959). Superovulating rabbits had an average of 58 CL but litter sizes at term were comparable to those of controls (Adams, 1960). In the generally monotocous sheep, the normal litter size of one was increased to about 4 by superovulation (Robinson, 1951).

Although concentrations of progesterone were elevated on Day 14 of pregnancy, they were followed by the same precipitous fall characterizing normal pregnancy in the hamster (Baranczuk & Greenwald, 1974). This is the most likely explanation for the onset of parturition at the normal time on Day 16 since injection of as little as 0·1 mg progesterone on Day 15 has been shown to delay parturition (Siegel & Greenwald, 1975). Plasma progesterone concentrations were found to be elevated after induction of superovulation in gilts (Webel, Reimers & Dziuk, 1975), and in pseudo-pregnant rats (Horikoshi & Wiest, 1971), but not in pregnant rabbits (Polidoro & Black, 1970). The only other hormone differing from control values in the superovulating hamster was oestradiol-17 β and the differences were not associated with alterations in serum levels of LH or FSH (Table 2).

In the present study, the hormone concentrations were all determined for animals killed at 09.00 hours. It is possible that sampling at other times during the day might reveal significant fluctuations in LH or FSH which could account for the observed differences in serum oestradiol between the PMSG-treated and control hamsters. A more heretical interpretation of these findings is that progesterone may, in some circumstances, directly depress ovarian secretion of oestrogen without altering gonadotrophin levels. Work is now in progress to explore the latter possibility.

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