MECHANISMS OF MAINTENANCE OF CORPORA LUTEA IN PIGS AND RATS

P. H. SAMMELWITZ*, J. P. ALDRED† AND A. V. NALBANDOV

Department of Animal Science, University of Illinois, Urbana, U.S.A.

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Summary. Progesterone injected into pregnant pigs from the time of ovulation until Days 10 to 13 of gestation does not prevent formation of corpora lutea. Injections of progesterone beyond Days 12 to 16 of pregnancy, i.e. after corpus luteum formation has been completed, results in complete and rapid destruction of the formed corpora lutea.

In contrast, progesterone injected into rats throughout the entire pregnancy or pseudopregnancy has no visible or measurable effect on corpora lutea.

Since in both rats and pigs a luteotrophic hormone (LTH) is presumed to be involved in the formation and maintenance of corpora lutea, it appears that progesterone is able to inhibit LTH release and hence maintenance of corpora lutea during pregnancy, but not during the luteal phase of the cycle. The possible theoretical implications of these findings concerning the role of LTH in maintenance of corpora lutea and the mechanisms of release of LTH in rats and in pigs are discussed.

INTRODUCTION

The role of progesterone in implantation and in the maintenance of pregnancy is too well established to need further review. However, the mechanisms which determine whether corpora lutea shall be allowed to regress in normally cycling mammals or maintained, if the animal becomes pregnant, are still unclear.

The luteotrophic hormone (LTH) of hypophyseal origin postulated by Astwood (1941) was found to be prolactin in the rat (Evans, Simpson, Lyons & Turpeinen, 1941), and was shown to be the hormone capable of maintaining corpora lutea in hypophysectomized rats. But prolactin is not luteotrophic in rabbits, guinea-pigs, women, cows and sheep (Moore & Nalbandov, 1955). In rats, hypophysectomy does not lead to abortion (Pencharz & Long, 1933), while castration does. A luteotrophic substance was found in placentae of rats (Astwood & Greep, 1938) and guinea-pigs (Davies, Amoroso & Dempsey, 1959).

While the existence of a luteotrophic hormone in species in which prolactin is not luteotrophic is highly probable, the controlling mechanisms which determine whether LTH should be secreted to maintain corpora lutea in pregnancy, or not secreted to allow corpora lutea to degenerate during normal cycles, are unknown.

* Department of Animal and Poultry Industry, University of Delaware, Newark, Delaware, U.S.A.

† Department of Animal Science, Purdue University, Lafayette, Indiana, U.S.A.

In pigs, progesterone can under certain circumstances completely destroy existing corpora lutea (Sammelwitz, Dziuk & Nalbandov, 1956; Sammelwitz & Nalbandov, 1958; Spies, Zimmerman, Self & Casida, 1958, 1959), but in rats the injection of as much as 8 mg of progesterone per day throughout pregnancy had no measurable or visible effect on the size and number of lifespan of corpora lutea (Sammelwitz *et al.*, 1956).

In the experiments reported here, the question was investigated why progesterone was luteolytic in pigs but not in rats, whether its luteolytic effect was due to its action on hypophyseal LTH or whether it was acting directly on the corpora lutea and, finally, whether there is a difference between pigs and rats in the source of LTH. These questions were studied in a series of experiments which are the subject of this paper.

MATERIALS AND METHODS

Mature female rats were placed with fertile males and daily vaginal smears were taken. Presence of spermatozoa in the smear was taken as evidence of mating. The mated females were randomly allotted to one of four groups, which included an uninjected control group and three groups which received subcutaneous injection of 4, 6 or 8 mg of progesterone daily. In order not to interfere with descent of eggs, injection began 2 days after mating (Day 3 of cycle) and continued until Day 19 of gestation when autopsy was performed. The ovaries were cleaned and weighed and the number and appearance of corpora lutea was determined.

The pigs used were young virgin animals which were purchased at the age of 4 to 5 months. They were checked daily for heat and treatments were not begun until it was established that the intervals between at least two successive heats fell within the normal range of about 21 days. Animals destined to be bred were allowed to mate on the 1st day of heat of the third oestrous cycle after the females had been placed on experiment.

Crystalline progesterone was dissolved in corn oil, the total dose of hormone to be injected being contained in 2 ml of solvent. Injections were made daily either intramuscularly or subcutaneously. Since there was no significant difference in the results obtained between the two routes of administration the data were combined.

Counting the 1st day of heat as Day 1 of the cycle, all treatments with progesterone were begun on Day 2, and autopsy was performed on Days 6, 10 or 13 of the cycle in non-pregnant female pigs, and on Days 7, 12, 22 or 28 of pregnancy in animals bred to fertile boars.

At autopsy the ovaries were removed, weighed and described morphologically (number and size of corpora lutea and follicles), and saved for histological examination. The proportion of functional luteal cells was estimated according to the method of Foley & Greenstein (1958). In some experiments, the forming or formed corpora lutea were separated from the ovarian stroma and the luteal tissue and ovarian stroma (including follicles) were weighed separately. In pigs forming corpora lutea shortly after ovulation are, as a rule, filled with lymph. In these forming corpora lutea, the liquid was separated from the already

solid luteal tissue, and the weights of luteal tissue and lymph were determined by difference. Also at autopsy, the number and condition of foetuses was noted and recorded.

RESULTS

Progesterone injected into pregnant rats at the rates of 4, 6 or 8 mg daily from Day 3 to Day 19 of gestation had no visible or measurable effect on ovarian weight, the number or size of corpora lutea, the number of living young or the implantation rate (Table 1).

TABLE 1
REPRODUCTIVE PERFORMANCE OF RATS INJECTED WITH
PROGESTERONE FROM DAY 3 TO 19 OF PREGNANCY

Progesterone (mg/day)	No. rats	Average No.		Implantation rate (%)	Average weight of ovaries (mg)
		Corpora lutea	Living foetuses		
Control	11	14.9	12.4	83.2	110.8
4	7	14.7	11.9	80.6	110.6
6	10	14.6	12.1	82.9	115.4
8	11	14.6	12.0	82.0	114.5

In the initial experiment involving pigs, progesterone was injected at four different levels into pregnant females which were killed 23 to 28 days after breeding. It was found (Table 2) that there was a progressive destruction of

TABLE 2
REPRODUCTIVE PERFORMANCE OF PIGS INJECTED WITH PROGESTERONE
FROM DAY OF MATING UNTIL DAYS 23 TO 28 OF PREGNANCY

Progesterone (mg/day)	No. gilts	Average No. $\pm$ S.D.		Implanted (%)
		Corpora lutea	Living foetuses	
0	9	12.2 $\pm$ 1.3	11.2 $\pm$ 1.4	92
50	6	10.3 $\pm$ 1.1	9.0 $\pm$ 1.7	87
	1	0.0	0.0	—
100	4	12.5 $\pm$ 1.3	10.0 $\pm$ 3.8	80
	3	5.7 $\pm$ 7.2*	6.0 $\pm$ 6.2	106
	2	0.0	0.0	—
200	4	13.3 $\pm$ 1.3	9.2 $\pm$ 3.6	70
	4	14.5 $\pm$ 3.7*	11.3 $\pm$ 3.0	78
	2	0.0	0.0	—
400	4	0.0	4.5 $\pm$ 2.7	?
	3	0.0	0.0	—

* Morphologically abnormal.

corpora lutea with increasing dosage of progesterone so that in the seven animals receiving 400 mg daily, no corpora lutea were visible at autopsy. At the lower doses of hormone (50 and 100 mg), considerable individual variations in the degree of luteolysis occurred between animals, between the two ovaries of single animals, and even between corpora lutea of a single ovary. In the cases in which progesterone treatment did not decrease the number of corpora lutea, their appearance, in comparison to corpora lutea of control animals, was significantly altered in that they were distinctly smaller, paler and resembled corpora albicantia.

After this initial experiment, the question arose whether progesterone acted destructively only on formed corpora lutea or whether it was able to prevent their formation. In subsequent experiments, progesterone-treated animals were killed throughout the interval during which corpora lutea were still being formed as well as at stages when normally they should have reached maximal

TABLE 3
EFFECT OF DAILY INJECTION OF 400 MG OF PROGESTERONE
(BEGUN ON DAY OF MATING) ON FORMATION OF CORPORA
LUTEA OF PREGNANT PIGS KILLED AT VARIOUS INTERVALS
AFTER MATING

<i>Time of autopsy (days pregnant)</i>	<i>No. gilts</i>	<i>Ovary weight $\pm$ S.D. (g)</i>	<i>No. corpora lutea $\pm$ S.D.</i>	<i>Functional lutein cells (%)</i>
7	4	15.4 $\pm$ 5.3	12.3 $\pm$ 0.5	64
12	4	15.3 $\pm$ 1.3	12.5 $\pm$ 3.7	62
22	4	10.7 $\pm$ 2.4*	9.5 $\pm$ 6.5†	50
22 Control	4	16.8 $\pm$ 3.0	15.8 $\pm$ 1.5	72

* Compared to control and Day 12: $P < 0.05$.

† Compared to control: $P < 0.01$; morphologically abnormal.

development. In this experiment, bred pigs were injected with progesterone at the rate of 400 mg/day beginning on the day following mating and animals were killed at random on Days 7, 12, or 22 of gestation. The results of this experiment (Table 3) showed that at neither 7 nor 12 days after breeding and after initiation of progesterone treatment was there any decrease in ovarian weight or in the number of corpora lutea, all of which appeared normal. However, 22 days after the first injection there was a significant reduction in ovarian size and in the number and size of corpora lutea which by this time had also become morphologically distinctly abnormal in comparison to corpora lutea of untreated control animals (Table 3).

In the final experiment in this series, the ability of progesterone to modify the formation of corpora lutea during the normal oestrous cycle was tested by injecting unbred females with 200 or 400 mg of progesterone daily beginning on Day 2 of the cycle. Because the effects of these two doses were not significantly different, the results obtained were combined (Table 4). Ovulation in pigs

occurs 30 to 40 hr after onset of heat and the first progesterone injection was given about the time of ovulation. The first group of animals was killed on Day 6 of the cycle when most of the collapsed follicles are fluid-filled sacs with rather thin and folded walls which show beginning luteinization. By Day 10, when the second group of animals was autopsied, most of the corpora lutea have become solid structures although some of them even at this stage in development still contain fluid.

In this experiment, even after 13 continuous days of treatment with high dosages of progesterone, no significant reduction in the number of corpora lutea or in the average weight of the luteal tissue per corpus luteum was noted (Table 4). All at 3 sampling days, there was a significant reduction in total ovarian weight due to a reduction in follicle number and in follicle size. There

TABLE 4

EFFECT OF DAILY INJECTIONS OF PROGESTERONE (BEGUN ON DAY OF MATING) ON OVARIAN COMPONENTS OF NON-PREGNANT PIGS AT THREE STAGES OF CYCLE

Time of autopsy (day of oestrous cycle)	Treatment	No. gilts	Average weight $\pm$ S.D. of				No. corpora lutea ($\pm$ S.D.)
			Ovaries (g)	Luteal tissue and fluid			
				Total (g)	Per corpus luteum (mg)	All ovarian fluid (g)	
6	Control	3	16.0 $\pm$ 3.4	4.5 $\pm$ 0.2	283 $\pm$ 42	5.6 $\pm$ 1.8	16.0 $\pm$ 0.6
	Treated	6	12.1 $\pm$ 6.6*	4.9 $\pm$ 2.7	331 $\pm$ 137	3.6 $\pm$ 3.8	14.7 $\pm$ 2.8
10	Control	4	30.7 $\pm$ 13.3	9.9 $\pm$ 2.5	533 $\pm$ 44	13.2 $\pm$ 9.6	18.5 $\pm$ 3.7
	Treated	6	19.6 $\pm$ 6.2†	7.9 $\pm$ 2.4	474 $\pm$ 100	6.0 $\pm$ 4.0	16.7 $\pm$ 3.6
13	Control	4	21.2 $\pm$ 4.5	9.3 $\pm$ 0.7	561 $\pm$ 77	5.9 $\pm$ 3.4	16.5 $\pm$ 3.0
	Treated	6	15.9 $\pm$ 3.6†	7.3 $\pm$ 1.4*	490 $\pm$ 62	3.5 $\pm$ 1.2	14.8 $\pm$ 2.7

* $P < 0.05$.

† $P < 0.01$ compared to its control.

is also a decrease in the total weight of ovarian fluid which on Days 10 and 13 is almost completely due to a decrease in follicular fluid. The only significant reduction in total luteal tissue weight was noted on Day 13, but the average weight of luteal tissue per corpus luteum was not significantly lower.

DISCUSSION

The data presented show that high doses of progesterone, when injected into pregnant pigs, either completely destroy formed corpora lutea or severely damage them morphologically and histologically. However, progesterone injected into pigs during the period of corpus-leutum formation is incapable of preventing their formation and exerts its destructive effect in either bred or unbred females only on fully formed corpora lutea, e.g. those normally present in pregnant females.

Presumably the pituitary gland of pigs produces a luteotrophic hormone

(LTH) which is responsible for the maintenance of corpora lutea and the results obtained can be ascribed to a deficiency of LTH in progesterone-injected pigs. If this assumption is correct, then the question arises why progesterone injection does not block the secretion of luteotrophin during the initial stages of corpus luteum formation and why this treatment is unable to prevent the growth and development of luteal tissues during the first 13 to 15 days of the cycle or of pregnancy.

Daily injection of high doses of progesterone throughout gestation or pseudopregnancy into rats was unable to affect either the lifespan or the morphological appearance of corpora lutea during pregnancy, nor was it able to prevent formation of corpora lutea, or to shorten the duration of pseudopregnancy (Sengar, 1959). If it is accepted that in rats corpora lutea depend on LTH for maintenance, then the data presented show that there is a species difference in the ability of progesterone to inhibit LTH secretion, or that the corpora lutea of pregnant and pseudopregnant rats, once their development is initiated, do not depend on LTH for support.

It appears possible that in both species a single outpouring of hypophyseal luteotrophin takes place at about the time of ovulation. According to this postulate, no further luteotrophic support would be needed to maintain corpora lutea for their allotted lifespan during the normal cycle in the pig or for the duration of pseudopregnancy in the rat. This would explain why it is impossible to block initial corpus-luteum formation in either the normally cycling pig, the bred pig, or the pregnant or pseudopregnant rat.

If the pig becomes pregnant, additional support for the maintenance of corpora lutea during gestation must be provided and the data presented suggest that additional release of LTH does occur. This may be accomplished in the pig by a neurohumoral signalling system which may be activated by the events of implantation occurring in the uterus about 15 days after mating. Since uterine contents of sheep (Moore & Nalbandov, 1953) and of cows (Hansel, 1959) are known to modify the lifespan of corpora lutea, uterine contents may initiate a neural signal to the pituitary gland which may result in the release of additional luteotrophin, which may continue to be released for the duration of pregnancy. This secondary outpouring of hypophyseal LTH can be inhibited by progesterone, resulting in the regression of formed corpora lutea.

In rats, the initial outpouring of LTH may be sufficient to initiate the growth and development of corpora lutea which are then able to function for the duration of pseudopregnancy. If the mating which induced the initial outpouring of LTH was fertile, secondary support for continued maintenance of corpora lutea beyond the normal duration of pseudopregnancy may come from extra-pituitary sources. The placenta is known to contain a substance capable of prolonging the lifespan of corpora lutea (Astwood & Greep, 1958; Davies, *et al.*, 1959). That corpora lutea may continue to function in the absence of the pituitary gland was shown by Pencharz & Long (1933) and others who have found that hypophysectomy does not terminate pregnancy in rats while ovariectomy does. However, a single injection of prolactin, which is usually assumed to be the LTH of rats, maintains corpora lutea of rats only for a few days and certainly not for the duration of pseudopregnancy.

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