J. Endocr. (1980), **84**, 101–108 *Printed in Great Britain*
0022–0795/80/0084–0101 $02.00/0

IN-VITRO STEROIDOGENESIS OF NEWLY FORMED CORPORA LUTEA AND THE NON-LUTEAL OVARY IN THE RAT, RABBIT, HAMSTER AND GUINEA-PIG

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(*Received* 1 *March* 1979)

SUMMARY

The steroidogenic abilities of the newly formed corpus luteum (8–10 h after ovulation) and the non-luteal ovary were compared in the guinea-pig, hamster, rabbit and rat using an in-vitro incubation technique. Histologically, newly formed rat corpora lutea (CL) were highly luteinized whereas the CL of the rabbit and guinea-pig were only partially luteinized. The CL of the hamster showed the least amount of luteinization.

Serum progesterone was highest in the rat (18 ± 3 (S.E.M.) ng/ml). In the hamster, it was about 8 ng/ml, whereas in the rabbit and guinea-pig it was about 1 ng/ml. Serum androstenedione ranged between 0·5 and 1 ng/ml. Serum testosterone was lowest in the hamster (60 pg/ml) and highest in the rabbit (470 pg/ml), whereas in the rat and guinea-pig, testosterone levels were similar (about 240 pg/ml). Serum oestrogens were at baseline levels in all species.

The CL of the rat exhibited considerably greater steroidogenic ability than the CL of the other species, producing 70 ± 6 ng progesterone/mg per h, 215 ± 14 pg androstenedione/mg per h, 49 ± 3 pg testosterone/mg per h, 3 pg oestrone/mg per h and 1 pg oestradiol/mg per h. Rabbit CL produced only progesterone (7 ± 2 ng/mg per h). Newly formed hamster CL produced none of the above steroids. In general, the ability of the CL to produce progesterone *in vitro* correlated with the degree of luteinization found by histological observation. Guinea-pig CL were embedded deeply in the ovary and could not be obtained without damage. Consequently, a portion of the ovary containing a corpus luteum was incubated. There was no difference in the steroid production by this portion of the ovary compared with the non-luteal ovary.

The non-luteal ovary of the rat produced the highest amount of progesterone (10 ± 2 ng/mg per h). The guinea-pig non-luteal ovary produced about 5 ± 2 ng progesterone/mg per h, whereas the non-luteal ovary of the rabbit did not produce any. On the other hand, the hamster non-luteal ovary lost progesterone. Non-luteal ovaries from all species produced androgens. The non-luteal ovary of the guinea-pig contained especially large numbers of atretic antral follicles. The guinea-pig non-luteal ovary produced extremely large amounts of androstenedione (1110 ± 210 pg/mg per h) and testosterone (606 ± 154 pg/mg per h) compared with the amounts produced by the non-luteal ovary of the rat, hamster and rabbit. In the non-luteal ovary, interstitium and atretic antral follicles are the probable source of androgens.

Oestrogen production by the non-luteal ovary was at baseline levels in the four species studied correlating with the absence of healthy antral follicles. The results indicate the extreme species differences that exist in ovarian function in the early postovulatory period.

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INTRODUCTION

The corpus luteum, a characteristic feature of the mammalian ovary, is known to produce mainly progesterone and in a few species oestrogens. However, the time needed for the formation of a well-defined corpus luteum (or one with the ability to secrete progesterone) after ovulation differs in various species. In the monkey and man, levels of serum progesterone do not rise until about 2 days after ovulation (Knobil, 1973). Similarly, levels of serum progesterone in the rabbit (Miller & Keyes, 1975) and guinea-pig (Croix & Franchimont, 1975) are very low on the morning of ovulation. On the other hand, levels of serum progesterone in the rat are already very high within a few hours of ovulation (Butcher, Collins & Fugo, 1974). A recent study in this laboratory has shown that corpora lutea of the hamster are unable to produce progesterone *in vitro* if removed on the morning of ovulation (09.00 h) (Terranova, Connor & Greenwald, 1978), whereas corpora lutea removed at a later time (15.00 h) are able to produce significant quantities of progesterone (K. Taya & G. S. Greenwald, unpublished observation). This observation led to a comparative study of the in-vitro steroidogenic ability of the newly formed corpora lutea (removed 8–10 h after ovulation) of the rat, rabbit, hamster and guinea-pig. In addition, the steroidogenic ability of the non-luteal ovary was also studied in these species. The present comparative study was thus undertaken to determine the ability of the newly formed corpus luteum compared with the non-luteal ovary of the above four species to produce different steroid hormones on the morning of ovulation. The hamster was included in this study for two reasons, to compare and contrast with other species and to confirm the previous report of Terranova *et al.* (1978).

MATERIALS AND METHODS

Guinea-pigs, hamsters, rabbits and rats were maintained in separate rooms at 22 °C on a 14 h light : 10 h darkness schedule (lights on at 05.00 h). The day of ovulation was determined in the guinea-pigs, hamsters and rats by studying the vaginal smears. In the rabbits, ovulation was induced by mating. Animals were killed on the morning of ovulation at 10.00 h by decapitation (guinea-pig, hamster and rat) or by cervical dislocation (rabbit). Before killing the rabbits, blood was collected from the ear vein. Blood from all the animals was allowed to clot overnight at 4 °C and the serum was then collected for radioimmunoassay of progesterone, androstenedione, testosterone, oestrone and oestradiol. The ovaries were excised immediately after killing and placed in ice-cold (4 °C) 0·9% saline until required for incubation. The oviducts were flushed to confirm the presence of tubal ova surrounded by granulosa cells. The corpora lutea of the hamsters, rabbits and rats were separated from the non-luteal ovarian remnants by dissection. Corpora lutea were difficult to obtain from guinea-pigs without contamination from the adhering interstitium, therefore, an ovarian segment, with and without a corpus luteum, was used. Tissues were blotted and weighed and divided into two groups. One group (three to five corpora lutea and a 10–20 mg piece of non-luteal ovary) was placed in 0·4 ml 95% ethanol within 30 min of removal from the peritoneal cavity for 'zero' time steroid determinations (initial concentration). The remaining tissues (three to five corpora lutea and a 10–20 mg piece of non-luteal ovary) were incubated separately in 1 ml freshly gassed (95% O_2 : 5% CO_2) Krebs–Ringer bicarbonate medium (Umbreit, Burris & Stauffer, 1953) for 2 h at 37 °C. After incubation, media were rapidly frozen using solid CO_2 and the tissues were stored in ethanol until required for steroid assays. Ovaries fixed in Bouin's fluid were used for histological study.

Steroid assays

Progesterone antisera (Surve, Basco, Brinckerhoff & Kirsch, 1976), androstenedione antisera (provided by Dr J. Resko, Oregon Regional Primate Research Center, Beaverton, Oregon, U.S.A.), testosterone antisera (Pang & Johnson, 1974), oestrone antisera (Wright, Collins & Preedy, 1973) and oestradiol antisera (Exley, Johnson & Dean, 1971) were

employed in the steroid determinations. The radioimmunoassay procedure and assay sensitivities were identical to those described previously (Saidapur & Greenwald, 1978, 1979; Terranova *et al.* 1978). Ether extracts of tissues, serum and incubation medium from each species exhibited parallelism in each steroid radioimmunoassay. Only those quantities on the linear portion of the parallel curve were used in steroid assays. The coefficients of variation for the intra-assay and interassay variances were respectively: 2·1 and 6·8% for progesterone; 1·8 and 5·9% for androstenedione; 4·2 and 7·3% for testosterone; 2·1 and 3·9% for oestrone; 1·8 and 3·9% for oestradiol. These calculations were based on duplicate determinations for six assays.

Statistics

To determine the significance of steroid production *in vitro*, Student's *t*-test was employed by comparing the mean tissue concentration of steroid before and after incubation. Differences were considered to be significant at $P < 0{\cdot}05$. Comparison of hormone levels or production rates between species was made by Duncan's multiple range test (Steel & Torrie, 1960).

RESULTS

Serum levels of steroid

On the morning of ovulation the highest serum progesterone level was found in the rat, but in the hamster these levels were approximately half (7–8 ng/ml) those found in the rat (Table 1). The lowest serum progesterone level was encountered in the rabbit and the guinea-pig. The serum androstenedione level was about the same in all species with the exception of the rabbit which exhibited the lowest ($P < 0{\cdot}01$) level. Serum testosterone levels were markedly raised ($P < 0{\cdot}01$) in the rabbit and low ($P < 0{\cdot}01$) in the hamster. The rat and guinea-pig had similar levels of testosterone and androstenedione. Serum oestrogen levels ranged between 5 and 15 pg/ml in all the species studied.

Table 1. *Levels of steroids in serum on the morning (10.00 h) of ovulation in the rat, rabbit, hamster and guinea-pig (values are means* ± S.E.M.; *numbers of animals in parentheses)*

Species	Progesterone (ng/ml)	Androstenedione (ng/ml)	Testosterone (pg/ml)	Oestrone (pg/ml)	Oestradiol (pg/ml)
Rat	18 ± 3 (7)	1·4 ± 0·1 (7)	234 ± 20 (7)	15 ± 2 (7)	10 ± 1 (7)
Rabbit	0·98 ± 0·2 (5)	0·38 ± 0·1 (5)	469 ± 64 (5)	12 ± 2 (5)	< 7·0 (5)
Hamster	7·8 ± 0·3 (7)	1·1 ± 0·1 (7)	60 ± 3 (7)	7 ± 2 (7)	10 ± 1 (7)
Guinea-pig	0·86 ± 0·1 (8)	1·3 ± 0·2 (8)	248 ± 30 (8)	15 ± 1 (8)	< 7·0 (8)

Luteal steroidogenesis in vitro

The corpora lutea of the hamster did not synthesize significant quantities of any steroid (Fig. 1). Rabbit corpora lutea produced only progesterone and of the five rabbits used, the corpora lutea of three produced small quantities of androgens and the corpora lutea of the remaining two lost both androstenedione and testosterone *in vitro*; on the average, therefore, no androgens were produced by the rabbit corpora lutea. On the other hand, the corpora lutea of the rat produced large quantities of progesterone, androstenedione and testosterone, whereas production of oestrone and oestradiol were at a baseline level (Fig. 1).

Non-luteal ovarian steroidogenesis in vitro

The non-luteal ovary of the hamster lost progesterone at a rate of 4·9 ng/mg per h, but produced significant quantities of androgen and oestrogen (Fig. 2). In contrast, the non-luteal ovary of the rabbit did not produce progesterone and oestradiol, although it produced

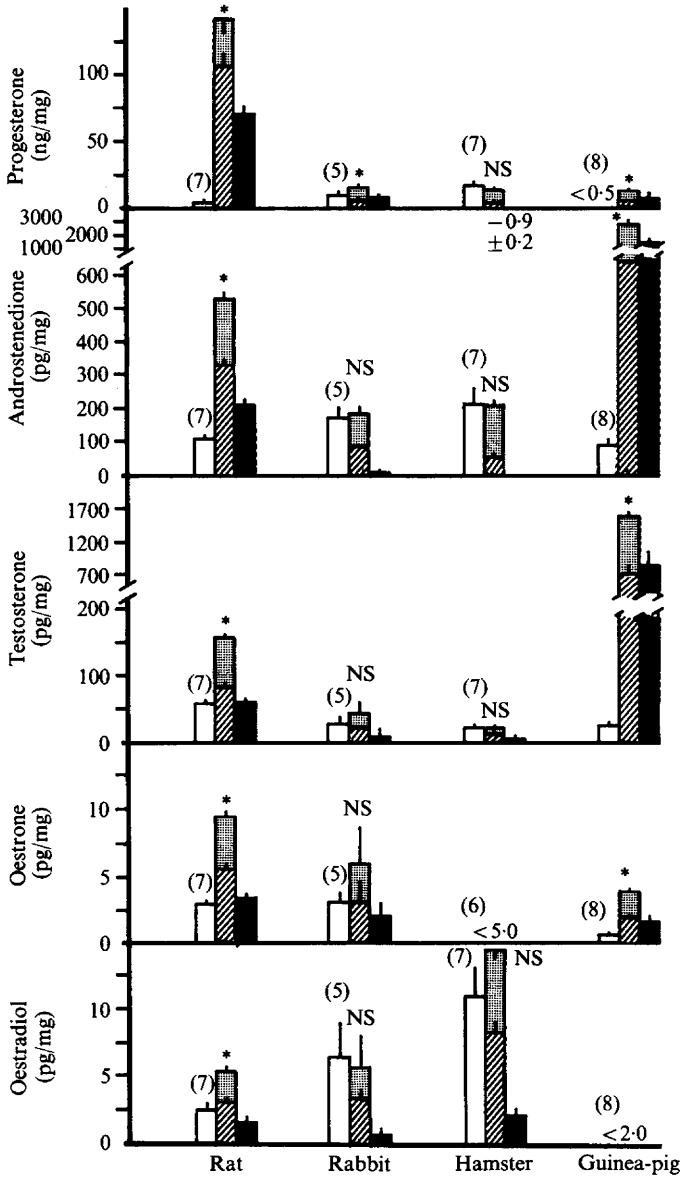

Fig. 1. Concentrations of progesterone, androstenedione, testosterone, oestrone and oestradiol in corpora lutea (before and after incubation) and incubation media and production rates (pg or ng/mg per h) *in vitro* for the rat, rabbit, hamster and guinea-pig on the morning (10.00 h) of ovulation. Only a portion of the guinea-pig ovary containing one corpus luteum was incubated. $*P<0{\cdot}05$: as compared with initial concentration; NS, not significant. The number of animals is shown in parentheses. Open bars, initial concentration; stippled bars, concentration in incubation medium; hatched bars, concentration in incubated tissue; solid bars, production rate per h. Production rate was derived by subtracting the initial concentration of steroid/mg tissue from the sum of the steroid concentrations in incubated tissue and its incubation medium. This value was then divided by two.

the other steroids. The non-luteal ovary of the rat produced the largest ($P<0{\cdot}05$) quantity of progesterone and also produced the other steroids (Fig. 2). The non-luteal ovary of the guinea-pig produced half as much progesterone as that of the rat and extremely large quantities of androstenedione (about 1000 pg/mg per h) and testosterone (600 pg/mg per h).

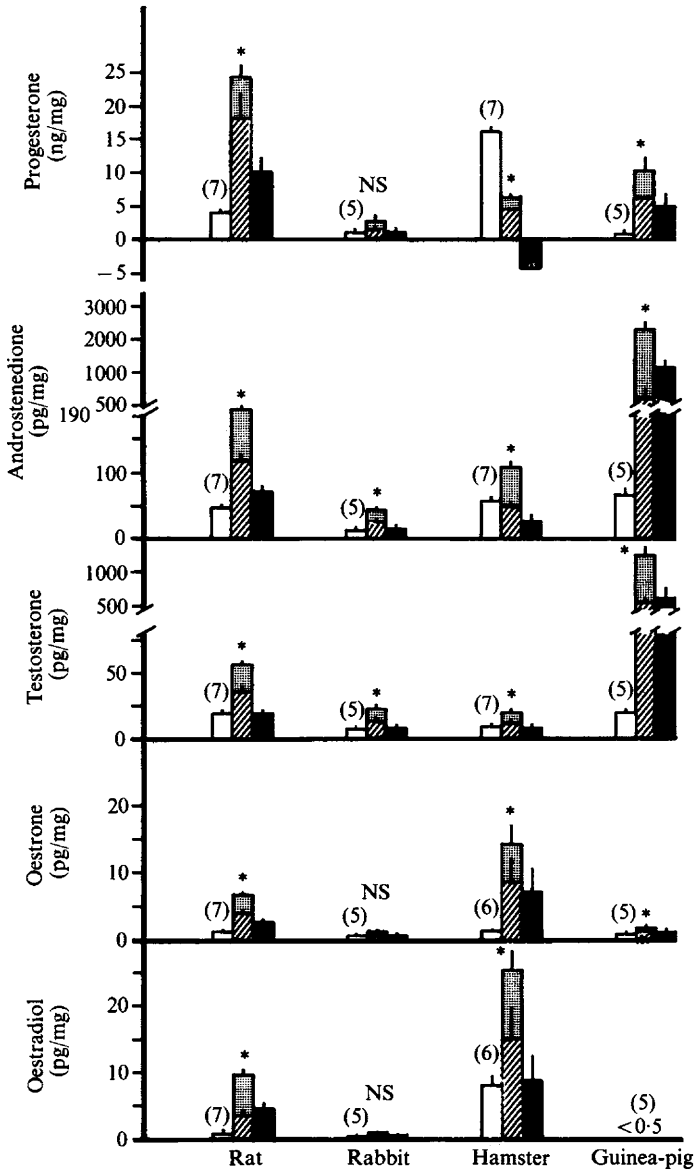

Fig. 2. Concentrations of progesterone, androstenedione, testosterone, oestrone and oestradiol in non-luteal ovary (before and after incubation) and incubation media and production rates (pg or ng/mg per h) *in vitro* for the rat, rabbit, hamster and guinea-pig on the morning (10.00 h) of ovulation. $*P < 0{\cdot}05$: as compared with initial concentration; NS, not significant. The number of animals is shown in parentheses. Open bars, initial concentration; stippled bars, concentration in incubation medium; hatched bars, concentration in incubated tissue; solid bars, production rate per h; calculated as described for Fig. 1.

However, the non-luteal ovary of the guinea-pig failed to produce oestradiol and only synthesized 1·5 pg oestrone/mg per h.

In the guinea-pig, ovarian segments with or without a corpus luteum produced similar quantities of progesterone, androstenedione and testosterone, and small quantities of oestrone, but neither compartment produced oestradiol.

Histology of the ovary

On the morning of ovulation, corpora lutea in the four species showed various degrees of luteinization as shown by their size and appearance. The luteal cells of the rat were by far the largest and were highly luteinized. In the rabbit and guinea-pig there was a large luteal cavity and only a few cells appeared to be luteinized. Thus, in the corpora lutea of the rabbit, guinea-pig and hamster both luteinized and non-luteinized granulosa cells were apparent. The ovaries of all species contained a few atretic antral follicles. However, the ovary of the guinea-pig contained particularly large numbers of atretic antral follicles.

DISCUSSION

This study examined in detail the in-vitro steroidogenic potential of the newly formed corpus luteum as opposed to the non-luteal ovary in four mammalian species. In addition to revealing the differences between the various species, the present study provided information on the nature of the steroids produced by these components of the ovary on the morning of ovulation.

Luteal steroidogenesis

Of the four species studied, a well-defined corpus luteum is formed by the morning of ovulation only in the rat. Previous ultrastructural studies have shown that granulosa cells in the rat begin to luteinize before ovulation (Björkman, 1962), but they do not luteinize in the rabbit (Blanchette, 1966) and the hamster (S. K. Saidapur, C. Bill & G. S. Greenwald, unpublished observation) where luteinization begins several hours after ovulation. Therefore, on the morning of ovulation, the luteal cells in the rabbit and hamster merely represent an intermediate stage in the formation of a well-defined corpus luteum. The present in-vitro findings on the production rate of progesterone by the corpus luteum of the rat, rabbit and hamster correlate well with their ultrastructural and histological differentiation as a steroidogenic tissue. For instance, the rat corpus luteum which is highly luteinized is also able to produce large quantities of progesterone (Fig. 1). On the other hand, the newly formed corpus luteum of the rabbit and hamster is poorly luteinized and consequently produces limited quantities of progesterone. In the hamster, corpora lutea removed later on the same day (15.00 h) show that large amounts of progesterone are produced *in vitro* (K. Taya & G. S. Greenwald, unpublished observations) correlating with the progressive increase in luteinization (S. K. Saidapur, C. Bill & G. S. Greenwald, unpublished observations).

A portion of the guinea-pig ovary incubated with or without the corpus luteum produced the same amount of progesterone suggesting, albeit indirectly, that the contribution by the corpus luteum at this time is negligible. This also correlates with the fact that the guinea-pig corpus luteum contained only a few luteinized and many non-luteinized granulosa cells. Secondly, serum progesterone was also very low (< 1 ng/ml) (Table 1). Further studies with the guinea-pig are needed to determine the true steroidogenic ability of the developing corpus luteum and the nature of its steroids.

The corpus luteum of the rat produced significant amounts of androstenedione and testosterone but the corpora lutea of the rabbit and hamster did not. Rat corpora lutea even produced small amounts of oestrogen, whereas in the rabbit and hamster luteal oestrogen production was negligible. Previous studies have shown that rabbit (Marchut, 1977) and hamster corpora lutea do not produce oestrogens (Terranova & Greenwald, 1978). Corpora lutea from the pregnant rat (Elbaum & Keyes, 1976) and the pregnant hamster can aromatize androgens to oestrogens (A. J. Vomachka & G. S. Greenwald, unpublished observations). Whether this is also true for the rabbit and guinea-pig is unknown.

In all species, except the hamster, there is a good correlation between levels of progesterone in serum and the ability of the new corpus luteum to produce progesterone *in vitro*. The situation in the hamster is enigmatic, since the corpora lutea and non-luteal ovary did not produce progesterone *in vitro*. This has been considered in detail recently by Terranova & Greenwald (1978).

Non-luteal ovarian steroidogenesis

The non-luteal ovaries of the rat and guinea-pig produced progesterone whereas the non-luteal ovaries of the rabbit and hamster did not. On the other hand, the non-luteal ovaries of all four species produced both androstenedione and testosterone although the production rate varied with the species. It is noteworthy that the non-luteal ovary of the guinea-pig produced extremely large quantities of both androstenedione and testosterone (Fig. 2). In sheep, atretic antral follicles are capable of producing androgens (Moor, Hay, Dott & Cran, 1977), but in the hamster, the interstitium appears to be a major source of testosterone (Saidapur & Greenwald, 1979). Histologically, large numbers of early atretic antral follicles were encountered in the guinea-pig ovary. Therefore, in view of the above findings, it is likely that atretic antral follicles in the guinea-pig ovary are the principal source of androgens on the morning of ovulation with the interstitium serving as an additional source of androgens in the guinea-pig. The non-luteal ovaries of the other species studied also contained a few atretic follicles and a moderate amount of interstitium, both perhaps contributing to the level of androgens.

It is well established that antral follicles are the main source of oestrogens in the mammalian ovary (review by Armstrong & Dorrington, 1977), but healthy antral follicles are rarely encountered on the morning of ovulation. Thus, the poor oestrogenic ability of the non-luteal ovary in these species correlates well with the absence of antral follicles. In general, levels of steroid hormones in serum observed on the morning of ovulation in the four species studied conform with previous reports (Butcher *et al.* 1974; Croix & Franchimont, 1975; Miller & Keyes, 1975; Saidapur & Greenwald, 1978; Terranova & Greenwald, 1978).

In summary, the present study has made a comparative assessment of the in-vitro steroidogenic ability of the corpus luteum and non-luteal ovary on the morning of ovulation in four mammalian species. It is evident that there is considerable species variation in the time needed for the completion of luteinization following ovulation, and the ability to produce various steroid hormones. The corpus luteum of the rat was the most precocious and versatile in steroidogenesis.

This research was supported by grants from the NIH (HD–00596) and The Ford Foundation. S. K. S. was supported by a postdoctoral fellowship of The Ford Foundation.

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