In PR rats, on days 8 and 12, the LH responses were as high as on day 8 of PSP and D-PSP. On day 22 of pregnancy, however, the responses were very much higher than on days 8 and 12. They were also higher than in PSP rats on day 12.

Discussion. Functional luteolysis – as indicated by the decrease and the increase of the plasma levels of P and DHP, respectively – occurs in the PSP rat between days 8 and 12[1,2] and in the PR rat between days 20 and 22[12,13]. Functional luteolysis is in both cases accompanied not only by resumption of follicle development, as indicated by the increase of the plasma levels of E_2, but also by a marked increase of the responsiveness of the pituitary gland to LHRH.

The present data confirm that in rats with a decidualized uterus luteolysis is postponed beyond days 8–12. In such rats there is no resumption of follicular development during that period. Furthermore, the increase in pituitary LHRH-responsiveness is also postponed. Our data are therefore in agreement with the suggestion that the pituitary LHRH-responsiveness does not increase as long as the CL are active.

The present data support the view that the physiological role of the decidua is the maintenance of CL function in case of pregnancy: a decidual luteotropin[14–17] may be the agent of the rescue of the CL from lysis. If this view is correct, then the role of the conceptus itself in the midpregnancy rescue of the CL is limited to the application of an essentially a-specific, mechanical stimulus for the progestational uterus (implantation of the blastocyst; see Introduction). Furthermore, if this theory is correct, there is an interesting parallel with the rescue of the CL following ovulation; in this case also the rescue is the result of a (neuroendocrine) chain of events which is initiated by a mechanical stimulus (copulation), applied by another individual (the male) and effectuated by an endocrine signal produced by the female herself (prolactin surges[8]).

Although the *state* of the CL – active or inactive – seems to be controlled by the female, it has been shown that the conceptus produces a luteotropin, presumably (rat) placental lactogen (rPL), which influences the production of P by the CL[18–20]. The present results agree with these data. On day 12, for instance there are clear-cut differences between D-PSP rats and PR rats: on that day the plasma levels of P are much higher in PR rats than in D-PSP rats, whilst the plasma levels of DHP are significantly lower.

The data presented in this study suggest that decidual luteotropin and rPL have different biological functions: decidual luteotropin may rescue the CL at midpregnancy, whilst rPL may regulate the *level* of activity of the rescued CL.

1 Koiter, T.R., Pols-Valkhof, N., van der Gugten, A.A., van Eekeren, J., and Schuiling, G.A., Acta endocr. *99* (1982) 31.
2 Schuiling, G.A., van der Gugten, A.A., Pols-Valkhof, N., and Koiter, T.R., Neuroendocrinology *41* (1985) 52.
3 Velardo, J.T., Anat. Rec. *122* (1955) 479.
4 Yochim, J.M., and DeFeo, V.J., Endocrinology *71* (1962) 134.
5 Anderson, L.L., Bland, K.P., and Melampy, R.M., Rec. Prog. Horm. Res. *25* (1969) 57.
6 Bradbury, J.T., Brown, W.E., and Gray, L.A., Rec. Prog. Horm. Res. *5* (1950) 151.
7 Greef, W.J. de, and Zeilmaker, G.H., Endocrinology *95* (1974) 565.
8 Smith, M.S., McLean, B.K., and Neill, J.D., Endocrinology *98* (1976) 1370.
9 Schuiling, G.A., and Gnodde, H.P., J. Endocr. *71* (1976) 1.
10 Koiter, T.R., Pols-Valkof, N., and Schuiling, G.A., Acta endocr. *92* (1979) 28.
11 Steel, R.D.G., and Torrie, J.H., Principles and Procedures of Statistics. McGraw-Hill Co. Inc, New York 1960.
12 Pepe, G.J., and Rothchild, I., Endocrinology *95* (1974) 275.
13 Taya, K., and Greenwald, G.S., Biol. Reprod. *25* (1981) 692.
14 Gibori, G., Rothchild, I., Pepe, G.J., Morishige, W.K., and Lam, P., Endocrinology *95* (1974) 1113.
15 Gibori, G., Basuray, R., and McReynolds, B., Endocrinology *108* (1981) 2060.
16 Gibori, G., Kalison, B., Warshaw, M.L., Basuray, R., and Glaser, L., Endocrinology *116* (1985) 1784.
17 Basuray, R., Jaffe, R., and Gibori, G., Biol. Reprod. *28* (1983) 551.
18 Kato, H., Morishige, W.K., and Rothchild, I., Endocrinology *105* (1979) 846.
19 Voogt, J., Robertson, M., and Friesen, H., Biol. Reprod. *26* (1982) 800.
20 Yogev, L., and Terkel, J., Acta endocr. *108* (1985) 305.

0014-4754/87/080895-04$1.50 + 0.20/0

Testosterone secretion by Mongolian gerbil interstitial cells during short-term incubation depends on androgen precursors and serum proteins but not on gonadotrophins

M. Fenske

Lehrstuhl für Tierphysiologie, Universität Bayreuth, D-8580 Bayreuth (Federal Republic of Germany), 8 October 1986

Summary. Interstitial cells from the testes of the Mongolian gerbil have been used to investigate the effects of serum proteins on testosterone production stimulated by hCG and steroidal precursors. Short-term incubation of interstitial cells with progesterone or DHEA resulted in a rapid increase of testosterone secretion; this effect was even more pronounced in the presence of calf serum. On the other hand, addition of hCG (10 mIU) had no significant effect on testosterone release during the 30-min incubation. These results demonstrate that the magnitude of the steroidogenic response of short-term incubated interstitial cells is a complex function, mainly of precursor concentrations and binding capacities of serum proteins but not of gonadotrophins.

Key words. Interstitial cells; Mongolian gerbil; in vitro secretion; testosterone; steroidal precursors; serum proteins.

Luteinizing hormone (LH) or human chorionic gonadotrophin (hCG) have been used to assess the secretory activity of interstitial cells of the testes. Using isolated interstitial cells, this steroidogenic response has been shown to provide a highly sensitive in vitro bioassay for the measurement of the biological activity of LH/hCG (rat[1–6], mouse[7–11], Mongolian gerbil[12–16]). A serious drawback of the assay is the interference caused by the presence of testosterone precursors in serum samples, which are rapidly converted to testosterone[3,10,16]. The interfering effects of these precursors are easily recognized by the fact that they elicit an apparent testosterone response which can be much greater than that induced by hCG[16]. In addition, hCG-stimulated testosterone secretion may be stimulated by the presence of serum macromolecules such as albumin or sex hormone binding globulin[17]. To test whether a similar effect of serum proteins can

Table 1. Effects of calf serum on testosterone production by Mongolian gerbil interstitial cells incubated with hCG, cortisol and steroidal precursors

	Testosterone secretion (ng/10^5 cells/30 min)	
	0.7 ml medium	0.4 ml medium + 0.3 ml serum
–	1.01 ± 0.04	0.92 ± 0.08
hCG	1.21 ± 0.07	0.94 ± 0.04
Cortisol	1.27 ± 0.03	1.21 ± 0.17
Progesterone	12.93 ± 0.73	26.07 ± 1.60*
Progesterone + cortisol	8.61 ± 0.48	19.58 ± 1.77*
DHEA	19.56 ± 1.87	41.37 ± 5.53*
DHEA + cortisol	18.71 ± 2.60	39.31 ± 3.07*

Isolated interstitial cells (10^5 cells/0.7 ml) were incubated at 35 °C for 30 min in the absence or presence of calf serum (0.3 ml), hCG (10 mIU) or steroids (1 µg). All values are the average of 6 determinations ± SEM. * $p < 0.01$.

Table 2. Basal and hCG- or steroidal precursor-stimulated testosterone secretion by Mongolian gerbil interstitial cells as a function of incubation length

a) 30-min incubation

	Testosterone secretion (% of controls)		
	0.7 ml medium (controls)	0.4 ml medium + 0.3 ml serum	0.1 ml medium + 0.6 ml serum
–	100 ± 13	102 ± 8	96 ± 2
hCG	100 ± 7	98 ± 5	88 ± 7
Progesterone	100 ± 9	306 ± 9*	262 ± 16*
DHEA	100 ± 7	310 ± 13*	342 ± 21*

b) 240-min incubation

	Testosterone secretion (% of controls)		
	0.7 ml medium (controls)	0.4 ml medium + 0.3 ml serum	0.1 ml medium + 0.6 ml serum
–	100 ± 4	199 ± 11*	199 ± 7*
hCG	104 ± 4	104 ± 4	96 ± 1

Interstitial cells (10^5 cells/0.7 ml) were incubated at 35 °C for 30 min (a) or 240 (b) in the absence or presence of calf serum (0.3 or 0.6 ml), hCG (10 mIU) or steroids (1 µg). All values are the average of 6 determinations ±SEM. * $p < 0.01$.

also be observed on precursor-stimulated testosterone secretion, interstitial cells derived from the testes of the Mongolian gerbil were short-term incubated without or with calf serum, steroidal precursors and/or saturating amounts of hCG.

Material and methods. Animals. Mongolian gerbils (*Meriones unguiculatus*, 6–10 months of age) from our colony were used. They were kept in single cages under controlled temperature (22 ± 1 °C), relative humidity (55%) and lighting conditions (light from 02.00 to 14.00), with food and water available ad libitum.

Substances. The first standard for human urinary hCG for bioassay (code No. 61/6) was used. The standard was obtained from the National Institute for Biological Standards and Control (Hampstead, London, England). Medium 199, fetal calf serum and HEPES were purchased from Serva (Heidelberg, FRG), 3-isobutylmethylxanthine, calf serum and non-radioactive steroids from Sigma (Munich, FRG). The medium for incubation contained 1% fetal calf serum, 25 mmol HEPES and 0.1 mmol 3-isobutyl-methylxanthine; pH was adjusted to 7.2 with 1.0 N NaOH.

Preparation and incubation of interstitial cells. Interstitial cells were prepared according to the method described in previous work[12,13]. Briefly, decapsulated testes of 6 animals were cut into thin pieces and were incubated for 25 min at 35 °C. After centrifugation (80 × g, 2 min), the pellet was gently resuspended in 40 ml medium and was filtered through a nylon gauze. Final concentrations of cells were 10^5 cells/0.7 ml or 10^7 cells/2 ml incubation medium. In the first set of experiments, 10^5 cells/vial were incubated with continuous shaking at 35 °C in the absence or presence of calf serum (0.3 or 0.6 ml), steroidal precursors (progesterone, DHEA: 1 µg), cortisol (1 µg) or hCG (10 mIU). In additional experiments, interstitial cells derived from testes of 6 animals were pooled, washed and divided into 6 equal portions, each containing 10^7 cells/vial; cells were incubated without secretagogues for 120 min, and 10-µl samples were withdrawn at 15-min intervals. To investigate the short-term effects of hCG or DHEA, 10^7 cells were incubated for 4 min in the absence of secretagogues, and were incubated for 6–12 min in the presence of hCG (10 mIU) or DHEA (1–4 × 500 ng); at 2-min intervals 5-µl samples were collected. At the end of the incubations cells were removed by centrifugation (8000 × g, 2 min) and the medium was stored at −24 °C. Unextracted 2-µl or 10-µl samples of the thawed medium were added directly to the testosterone assay[13].

Statistics. The significance of the observed differences was calculated using the Mann-Whitney U-test (independent groups) or the Wilcoxon matched pairs signed rank test (dependent groups). Differences were considered to be not significant if the calculated values exceeded the 5% probability value.

Results. In the absence of precursors or serum proteins, testosterone production by isolated cells was 1.01 ± 0.04 ng/10^5 cells/30 min. Addition of as much as 10 mIU hCG did not significantly stimulate testosterone secretion during the 30-min incubation (table 1). Compared to basal and hCG-dependent testosterone secretion, output was drastically elevated by the addition of progesterone or DHEA ($p < 0.01$). This effect was even more pronounced in cells incubated with precursors and calf serum. While the addition of cortisol had no significant effect on basal testosterone production, progesterone-stimulated testosterone secretion was reduced in interstitial cells incubated with cortisol, either in the absence or presence of calf serum. As summarized in table 2, basal and hCG-stimulated testosterone production was quite similar in interstitial cells which were incubated without or with 0.3 or 0.6 ml calf serum. In interstitial cells incubated over 240 min, basal but not hCG-stimulated testosterone secretion was significantly elevated by the addition of calf serum (0.3 or 0.6 ml: $p < 0.01$).

To measure the overall steroidogenic capacity of interstitial cells which can be derived from the testes of individual animals, 10^7 cells were incubated with 10 mIU hCG or 500 ng DHEA for 6–12 min. As illustrated in table 3, testosterone output was very low during the preincubation period (6–13 ng/10^7 cells) and increased linearly with the length of incubation (correlation coefficient r_{15-90} min = 0.96, $p < 0.001$). Production then ceased until the end of incubation. The presence of 10 mIU hCG had no stimulatory effect on testosterone production (basal secretion: 8 ± 3 ng/10^7 cells, hCG-stimulated secretion: 15 ± 3 ng/10^7 cells). Addition of DHEA (500 ng) drastically increased testosterone output ($p < 0.03$) within 2–5 min after DHEA addition. A further increase of testosterone production occurred when cells were repeatedly stimulated with DHEA pulses (4 × 500 ng: 813 ± 81 ng/10^7 cells/6 min, $p < 0.03$).

Discussion. The results of the present investigation are in close agreement with earlier work in the Mongolian gerbil showing that steroidal precursors are effectively converted to testosterone[15,16,18]. In contrast to interstitial cells of the mouse or rat[10,19], testosterone synthesis in Mongolian gerbil interstitial cells was maximally stimulated by DHEA (this study). As shown earlier[16], already 2 min after the addition of 500 ng DHEA, testosterone output was strongly elevated; in addition, secretion could be significantly stimulated by the addition of only 2 ng DHEA in cells incubated over 240 min. Taking into account the high serum levels of DHEA and

Table 3. Time-course of testosterone secretion by Mongolian gerbil interstitial cells under basal conditions (a) and after the addition hCG (b) or DHEA (c, d)

	Testosterone secretion (ng/10^7 cells)								
a) Basal secretion during prolonged incubation	Length of incubation (min) 15	30	45	60	75	90	105	120	
	13 ± 1	17 ± 3	28 ± 2	34 ± 3	43 ± 1	51 ± 3	53 ± 6	53 ± 5	
b) Single pulse of hCG	Length of incubation (min) −4	−2	0	2	4	6			
	12 ± 2	10 ± 1	8 ± 3	10 ± 2	13 ± 2	15 ± 3			
			↑ hCG added						
c) Single pulse of DHEA	Length of incubation (min) −4	−2	0	2	4	6	8	10	12
	6 ± 3	10 ± 4	6 ± 3	239 ± 62*	269 ± 13*	272 ± 24*	263 ± 7*	270 ± 14*	257 ± 11*
			↑ DHEA added						
d) Repeated pulses of DHEA	Length of incubation (min) −4	−2	0	2	4	6			
	13 ± 2	9 ± 1	10 ± 1	339 ± 25*	652 ± 47*	813 ± 81*			
			↑	↑ DHEA added	↑	↑			

After preincubation of cells for 4 min, hCG (10 mIU) or DHEA (1–4 × 500 ng) were added at time 0 (arrow heads). All values are the average of six determinations ± SEM. *$p < 0.03$.

other precursors in most species[20], it is not surprising that the addition of 50–100 µl serum will seriously interfere with the measurement of LH/hCG and result in an overestimation of gonadotrophins. In an attempt to eliminate these interfering factors, serum samples were subjected either to ether extraction or to charcoal adsorption prior to assay[8, 10]. Ether extraction was found to be ineffective, but charcoal treatment eliminated the source of non-parallelism of male plasma; however, this treatment resulted in a 25% decrease of LH bioactivity[10]. As shown recently[16], and in this work, the stimulatory effect of hCG and steroidal precursors on testosterone production by isolated cells could be determined separately by short-term incubation of cells with hCG or DHEA. While the presence of 100–500 ng DHEA induced a marked dose-dependent stimulation of testosterone secretion, hCG had no effect on steroidogenesis during the 30-min incubation (table 1). Therefore, it is advisable that this rapid in vitro test should be performed whenever precursor concentrations in serum samples may be high (e.g. after application of stressors[21] or ACTH administration[22], and where LH levels are likely to be low. If testosterone production by cells incubated with serum is significantly higher than basal production, hCG should be removed from samples either by acetone precipitation[23] or by adsorption to concanavalin[24].

As shown in this study, precursor-stimulated testosterone production can be further stimulated by the addition of calf serum. These are the first experimental results to confirm the earlier hypothesis of Eik-Nes[25] that testosterone secretion could be explained by the presence of plasma constituents, and the findings of Ewing et al.[17] that testosterone secretion and diffusion into blood is significantly enhanced in the presence of serum proteins. A similar non-gonadotrophic factor in male plasma has been postulated already by Lichtenberg and Pahnke[8] and Rajalakshmi et al.[10] but its nature had not been identified.

In summary, these results demonstrate that the magnitude of the steroidogenic response of interstitial cells incubated for a short time is a complex function, mainly of the levels of steroidal precursors and the binding capacity of serum proteins, but not of LH or hCG.

1 Dufau, M. L., Mendelson, C., and Catt, K. J., J. clin. Endocr. Metab. *39* (1974) 610.
2 Mendelson, C., Dufau, M., and Catt, K., J. biol. Chem. *250* (1975) 8818.
3 Garfink, J. E., Moyle, W. R., and Bahl, O. P., Gen. comp. Endocr. *30* (1976) 292.
4 Solano, A. R., Dufau, M. L., and Catt, K. J., Endocrinology *105* (1979) 372.
5 Verhoeven, G., Koninckx, and de Moor, P., J. Steroid Biochem. *17* (1982) 319.
6 Molenaar, R., Rommerts, F. F. G., and van der Molen, H. J., Int. J. Androl. *6* (1983) 261.
7 van Damme, M.-P., Robertson, D. M., Romani, P., and Diczfalusy, E., Acta endocr. *74* (1973) 642.
8 Lichtenberg, V., and Pahnke, V. G., Acta endocr., Suppl. *202* (1976) 54.
9 Schumacher, M., Schäfer, G., Holstein, A. F., and Hilz, H., FEBS Lett. *91* (1978) 333.
10 Rajalakshmi, M., Robertson, D. M., Choi, S. K., and Diczfalusy, E., Acta endocr. *90* (1979) 585.
11 Hunter, M. G., Magee-Brown, R., Dix, C. J., and Cooke, B. A., Molec. cell. Endocr. *25* (1982) 35.
12 Probst, B., and Fenske, M., Comp. Biochem. Physiol. *85* A (1986a) 263–271.
13 Fenske, M., and Probst, B., Endokrinologie *79* (1982) 190.
14 Probst, B., Acta endocr. *103* (1983) 567.
15 Fenske, M., Comp. Biochem. Physiol. *85* A (1986a) 263–271.
16 Fenske, M., Comp. Biochem. Physiol. *85* A (1986b) 273–279.
17 Ewing, L. L., Chubb, C. E., and Robaire, B., Nature *264* (1976) 84.
18 Fenske, M., J. steroid Biochem., submitted.
19 Zeillinger, R., and Spona, J., Endocr. exp. *18* (1984) 141.
20 Kime, D. E., Vinson, G. P., Major, P. W., and Kilpatrick, R., in: General, Comparative and Clinical Endocrinology of the Adrenal Cortex, p. 183. Eds I. Chester-Jones and I. W. Henderson. Academic Press, London 1980.
21 Goncharov, N. P., Taranov, A. G., Antonichev, A. V., Gorlushkin, V. M., Aso, T., Cekan, S. Z., and Diczfalusy, E., Acta endocr. *90* (1979) 372.
22 Tremblay, I., and Belanger, A., Steroids *44* (1984) 57.
23 Albert, A., Rec. Prog. Horm. Res. *12* (1956) 227.
24 Louvet, J. P., Nisula, B. C., and Ross, G. T., J. Lab. clin. Med. *86* (1975) 883.
25 Eik-Nes, K. B., Rec. Prog. Horm. Res. *27* (1971) 517.

0014-4754/87/080898-03$1.50 + 0.20/0