	Blood glucose (mmol l^{-1})	Growth rate (g day^{-1})	Cardiac output (ml·100 g^{-1}·min^{-1})	Mean blood pressure (mmHg)	Tibia blood flow (ml·100 g^{-1}·min^{-1})	Tibia length (mm)	Tibia weight (mg)
Controls (7)	6.25 ± 0.54	2.48 ± 0.42	28.6 ± 2.3	121 ± 7	26.5 ± 2.5	35.8 ± 0.3	554 ± 28
14-day diabetic (6)	25.1 ± 1.4[d]	−1.30 ± 0.45[d]	22.1 ± 2.1	113 ± 8	8.9 ± 1.0[d]	35.0 ± 0.4	480 ± 33
14-day diabetic+insulin (9)	8.9 ± 0.9[d]	2.86 ± 0.12[d]	30.3 ± 2.5[a]	124 ± 8	36.6 ± 2.9[d]	36.1 ± 0.3[a]	558 ± 29
Controls (8)	6.67 ± 0.78	2.25 ± 0.26	23.5 ± 1.9	119 ± 6	23.0 ± 2.4	37.6 ± 0.36	626 ± 22
28-day diabetic (10)	35.8 ± 1.9[d]	−0.96 ± 0.32[d]	28.7 ± 2.5	87 ± 6[c]	13.7 ± 2.2[b]	35.1 ± 0.24[d]	486 ± 25[d]
Controls (13)	6.94 ± 0.47	2.15 ± 0.17	21.7 ± 1.4	121 ± 4	22.6 ± 3.4	38.8 ± 0.21	677 ± 18
56-day diabetic (7)	29.6 ± 2.03[d]	−1.11 ± 0.39[d]	31.9 ± 4.1[b]	102 ± 10[a]	12.1 ± 1.7[a]	34.9 ± 0.26[d]	478 ± 0.24[d]

Values given are of mean ± SEM. The figures in parentheses are the numbers per group. The significance of differences between diabetic groups and their controls and between the insulin-treated diabetic group and the untreated 14-day diabetic group were assessed by t-tests, the probability values obtained being indicated by: [a] $p < 0.05$; [b] $p < 0.02$; [c] $p < 0.005$ and [d] $p < 0.001$.

days since no further depression was evident at 28 or 56 days. In no other tissue was such a marked reduction in blood flow seen. The reduced tibial blood flow was accompanied by a cessation of tibial growth whether measured by length or weight.

The insulin treated 14-day diabetic animals were found to have approximately 36% higher mean cardiac output than the untreated 14-day diabetic group. Since insulin appeared to have no major effect on mean blood pressure a reduction in peripheral resistance is indicated. Tibial blood flow was approximately four times higher in the insulin treated diabetic group than in the untreated 14-day diabetic group and about 38% higher than in the control group.

The results demonstrate a major reduction in tibial blood flow in intact anaesthetised streptozotocin diabetic rats after 14, 28 and 56 days. This is in agreement with data obtained in pithed animals[9]. The change was not due to a general circulatory alteration since cardiac output was not found to be reduced in any of the diabetic groups and was actually increased above that of the controls in the longest term diabetic animals. Similarly the dramatic increase in tibial blood flow resulting from insulin treatment was much too large to be accounted for by the more modest increase in cardiac output. The reversal by insulin of the depressed tibial blood flow of the 14-day diabetic rats strongly suggests that the depression in this diabetic group was due to insulin deficiency rather than to any direct effect of streptozotocin. It would seem likely that this also applies to the two longer term diabetic groups although this was not demonstrated by the present results.

The mechanisms of the above changes require further elucidation. Since depression of tibial flow has also been shown to occur in pithed rats[9] it would appear not to be mediated by autonomic mechanisms. It may be related to the reduced bone turnover which has been demonstrated in this model of diabetes[7,8].

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Decidua and the control of corpus luteum function, follicular development and pituitary LHRH-responsiveness in pseudopregnant and pregnant rats

G. A. Schuiling, N. Pols-Valkhof, G. C. J. van der Schaaf-Verdonk, F. H. de Jong* and T. R. Koiter

*Division of Reproductive Biology, Department of Obstetrics and Gynaecology, University of Groningen, 59 Oostersingel, NL-9713 EZ Groningen (The Netherlands), and *Department of Biochemistry II, Erasmus University of Rotterdam, PO Box 1738, NL-Rotterdam (The Netherlands), 12 February 1987*

Summary. The mid-pregnancy rescue of corpora lutea can be mimicked in the pseudopregnant rat by induction of decidual tissue in the uterus: in such rats, around day 10, there is neither luteolysis, nor resumption of follicle-development or increase of the pituitary responsiveness to LHRH. The results suggest that the mid-pregnancy rescue of corpora lutea is caused by a maternal factor.

Key words. Mid-pregnancy rescue of corpus luteum function; pseudo-pregnancy; pituitary LHRH-responsiveness; luteolysis; decidua.

Around day 10 of pseudopregnancy (PSP) the LHRH-responsiveness of the rat pituitary gland begins to increase. This increase coincides with functional luteolysis, that is, cessation by the corpora lutea (CL) of the production of progesterone (P) in favor of the production of the P-metabolite 20α-dihydroprogesterone (DHP)[1].

We demonstrated recently that luteolysis and increase of pituitary LHRH-responsiveness did not occur in PSP rats which were treated with exogenous P, if the treatment with P was initiated before the onset of luteolysis. These observations led to the suggestion that pituitary LHRH-responsiveness does not increase as long as the CL are active[2].

The relationship between CL activity and pituitary LHRH-responsiveness was further investigated using rats with CL whose life span was lengthened in a more physiological way by decidual cells which developed in the uterus from endometrial fibrocytic cells in response to blastocyst implantation[3] or (in PSP rats) in response to traumatization of the uterus[4]. For this purpose PSP rats with a decidualized uterus (D-PSP rats[5,6]) and pregnant (PR) rats were used.

Materials and methods. Three-month-old female Wistar rats with regular 4-day ovulatory cycles were used. Vaginal smears were taken daily. In one group of animals the cervix uteri was stimulated electrically at 17.00 h during pro-estrus and at 15.00 h during estrus[7]. Such a stimulation provokes the secretion of prolactin[8] necessary to 'activate' the newly-formed CL.

The first day following the last cervical stimulation was denoted day 1 of PSP. On day 4, in some of the PSP rats (D-PSP rats) both horns of the uterus were traumatized in order to evoke the development of decidual cells[4]; the other PSP rats were sham-operated. Another group of animals (PR

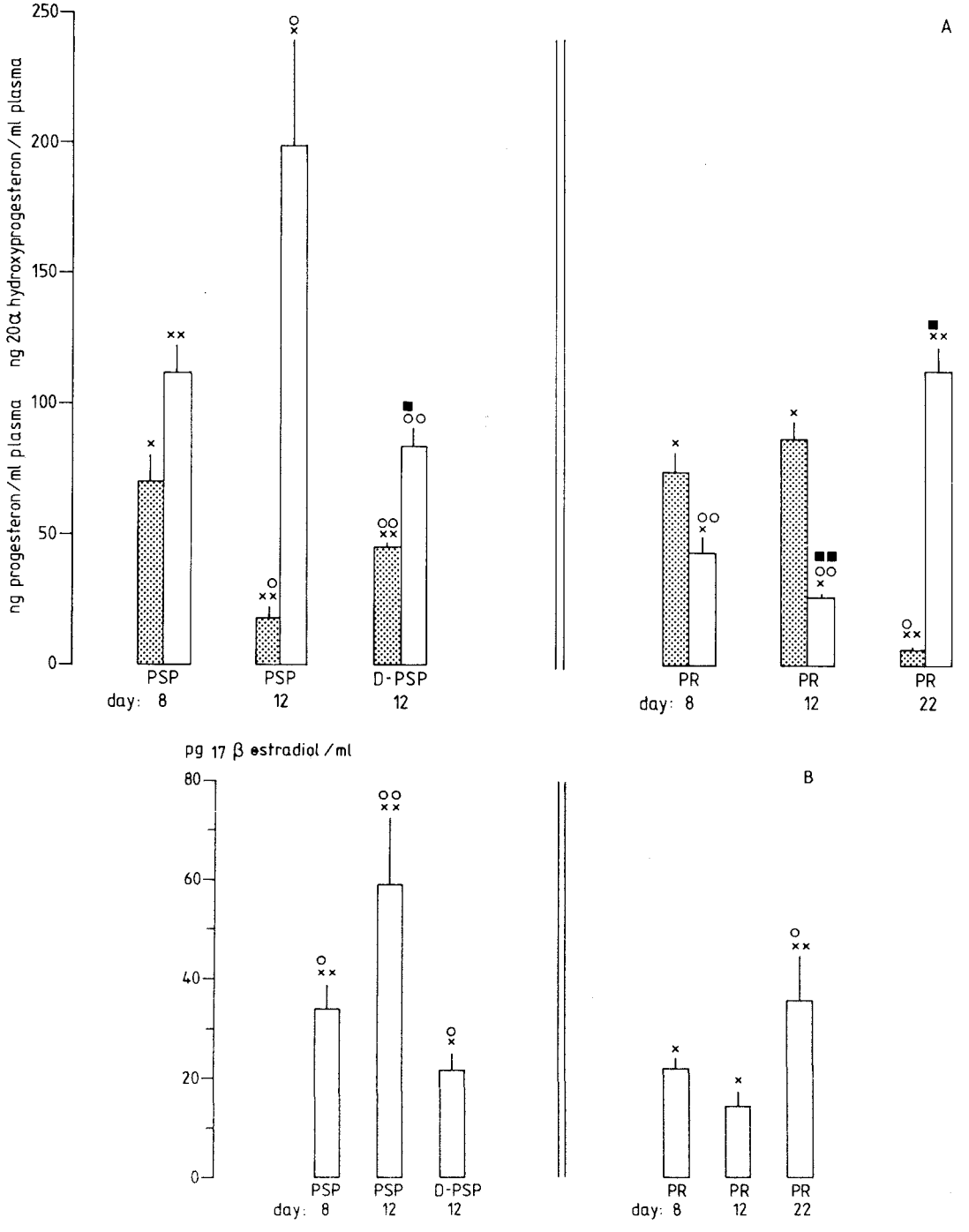

Figure 1. *A* Plasma progesterone (black bars) and 20α-dihydroprogesterone (open bars) concentrations; *B* plasma 17β-estradiol concentrations (mean ± SEM) for, *A* and *B*, pseudopregnant (PSP) rats, pseudopregnant rats with a decidualized uterus (D-PSP rats) and pregnant (PR) rats. No. of rats: see fig. 2. x-xx; o-oo: $p < 0.05$.

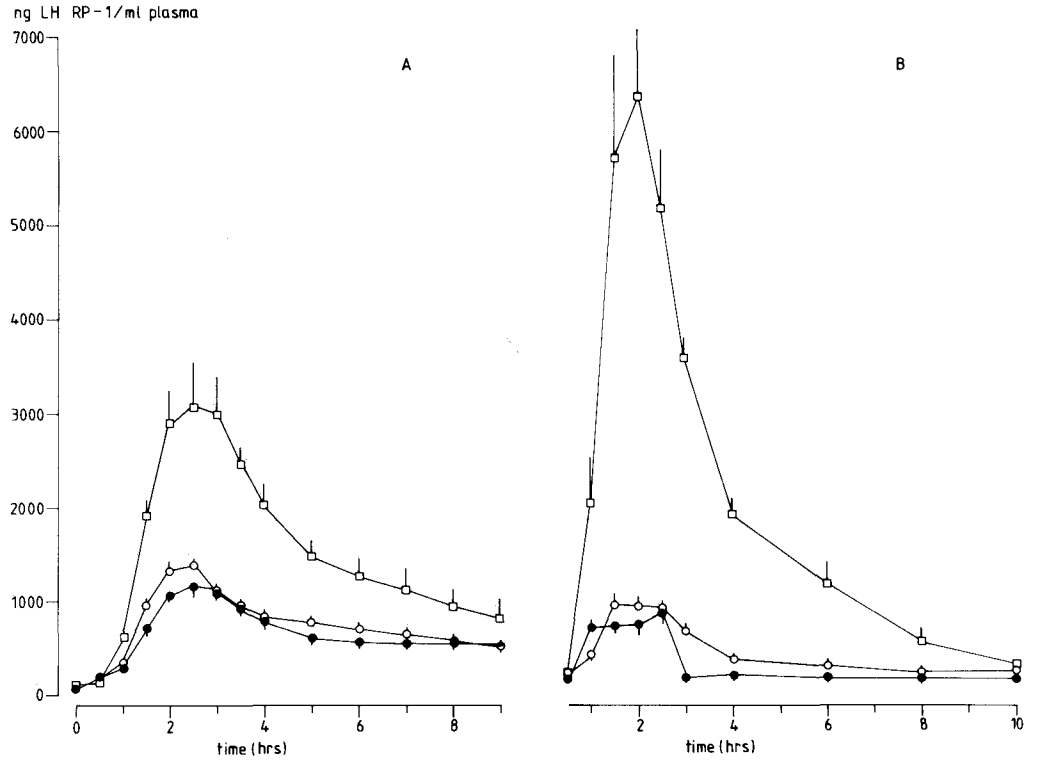

Figure 2. The course of changes in plasma LH concentrations (mean ± SEM) induced by constant rate infusion of LHRH (104 ng/h during 21 h) into pseudopregnant (PSP) rats, as well as into pseudopregnant rats with a decidualized uterus (D-PSP rats) and pregnant (PR) rats. Left panel: PSP rats. ○——○: day 8 (n = 13); □——□: day 12 (n = 6). D-PSP rats. ●——●: day 12 (n = 7). Right panel: PR rats. ○——○: day 8 (n = 5); ●——●: day 12 (n = 7); □——□: day 22 (n = 5).

rats) were mated to males of proven fertility. Only animals which exhibited sperm in the estrus vaginal smear were used. The day following estrus was denoted day 1 of pregnancy. Prolonged stimulation of LH secretion by the pituitary gland was effected by constant rate infusion of LHRH (104 ng/h during 21 h) via a cannula inserted into the right jugular vein[9]. The infusions were started at 12.00 h on days 8 and 12 of PSP and on days 8, 12 and 22 of pregnancy. During infusion of LHRH the animals were anesthetized with sodium phenobarbitone in order to damp the hypothalamic LHRH secretion which might induce a spontaneous LH surge on day 12 of PSP[10]. Rats first received an intraperitoneal injection of 80 mg/kg b.wt at 11.00 h and then another one of 30 mg/kg at 15.00 h.

During LHRH infusion a series of blood samples for assay of LH were taken from a cannula inserted into the right carotid artery. Samples for the determination of P, DHP and estradiol (E_2) were taken immediately before infusion of LHRH. During insertion of the cannulas (between 09.00–10.00 h) the rats were anesthetized with ether.

Plasma P and DHP concentrations were measured by radioimmunoassay at the Groningen Isotope Laboratory (Dr J. J. Pratt); plasma E_2 concentrations were measured by radioimmunoassay in the laboratory of one of the authors (FHdJ). Plasma LH concentrations were measured by double antibody radioimmunoassay with NIAMDDK-rat LH-RP-1 as reference preparation. The maximal height (ng LH/ml plasma) of the LH peaks induced by constant rate infusion of LHRH was considered to indicate the responsiveness of the pituitary gland to LHRH.

Statistical comparisons were made by analysis of variance and then by Duncan's multiple comparison test[11]. A difference was considered to be significant when the analysis of variance showed significant heterogeneity for the whole group and the multiple comparison gave a value of $p < 0.05$ for the two groups concerned.

Results. 1. Plasma P, DHP and E_2 concentrations. As shown in figure 1, in PSP rats the plasma levels of P were higher on day 8 than on day 12. The day-12 levels of D-PSP rats were in an intermediate position. The levels of DHP, on the other hand, were much higher in PSP rats on day 12 than on day 8, but in D-PSP rats they were as high on day 12 as on day 8. In PSP rats the plasma levels of E_2 were much higher on day 12 than on day 8. In D-PSP rats, on the other hand, the plasma E_2 levels were somewhat lower on day 12 than on day 8 of PSP. These changes in the plasma concentrations of P, DHP and E_2 indicate that in PSP rats but not in D-PSP rats, luteolysis and resumption of follicle development had occurred between days 8 and 12.

In PR rats the plasma levels of P were still as high on day 12 as on day 8. On day 22 they were markedly decreased. The levels of DHP were slightly higher on day 8 than on day 12, but on day 22 they were strongly increased. The levels of E_2 were as high on day 12 as on day 8. On day 22, however, they were increased. These data indicate that in PR rats luteolysis and resumption of follicle development had occurred on day 22.

On day 8, PR rats produce less DHP than PSP rats. On day 12, when the production of DHP by PSP rats had increased markedly, and that of D-PSP rats was still unchanged, PR rats produced even less DHP. On this day, however, PR rats produced more P than D-PSP rats.

2. LH responses. See figure 2. In PSP rats the LH responses induced by LHRH infusion were much higher on day 12 than on day 8. Responses induced on day 12 in D-PSP rats, on the other hand, were still as high as on day 8.

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.

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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