High-dose progesterone infusion in healthy males: evidence against antiglucocorticoid activity of progesterone

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High concentrations of unbound cortisol in late pregnancy have been explained by the antiglucocorticoid activity of high progesterone levels. To further test this hypothesis we studied the effect of high-dose progesterone on baseline and corticotrophin-releasing hormone (CRH)-induced hormone secretion in humans. In a double-blind crossover study eight healthy male volunteers received either progesterone (0.714 mg·kg⁻¹·h⁻¹ for 60 min followed by a dose of 0.45 mg·kg⁻¹·h⁻¹ over a total infusion time of 315 min) or vehicle as a continuous intravenous infusion. At 210 min a CRH test (0.1 μg/kg body weight as bolus iv) was performed. Within 30 min after the start of progesterone administration the serum progesterone level increased to 454 ± 31 nmol/l and remained in the range of third trimester pregnancy concentrations throughout the infusion period. During vehicle infusion the progesterone level remained in the normal range for healthy males and demonstrated a small but significant increase after CRH (1.52 ± 0.23 vs 0.74 ± 0.14 nmol/l; p < 0.01). However, baseline and CRH-stimulated serum cortisol and plasma adrenocorticotropic hormone remained unaffected by high-dose progesterone. Moreover, unbound salivary cortisol also was not affected by progesterone, suggesting that there is no significant competition for transcortin binding sites. In conclusion, no antiglucocorticoid activity was found after short-term administration of progesterone in males. These findings cast doubts on the concept that the alterations of the pituitary–adrenal axis in late pregnancy are induced by the antiglucocorticoid activity of high progesterone concentrations.

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Total serum cortisol and unbound cortisol increase during pregnancy (1–8). Moreover, in third trimester pregnancy the cortisol response to exogenously administered adrenocorticotropic hormone (ACTH) is enhanced (6). While the diurnal rhythm of unbound cortisol is preserved at a higher level (4), suppression of serum cortisol by dexamethasone is incomplete in late pregnancy (6). These observations suggest a relative refractoriness to glucocorticoid feedback in women in late pregnancy (4, 6, 7).

Progesterone has been shown to have antiglucocorticoid actions at steroid receptors in various tissues (9–11). In addition, progesterone has been reported to antagonize glucocorticoid inhibition of β-endorphin secretion from cultured pituitary cells (12). However, partial glucocorticoid agonist activity of progesterone has also been described (11, 13, 14). We have found that the increase in progesterone concentrations in late pregnancy correlated with the increase in salivary cortisol, suggesting that high progesterone concentrations have significant antiglucocorticoid activity also in vivo (4). In addition, in vivo studies in non-pregnant ewes have demonstrated that progesterone can interfere with the delayed feedback of cortisol (15).

The present study was undertaken to investigate the acute effect of late pregnancy progesterone concentrations on baseline and CRH-stimulated ACTH and cortisol secretion in humans.

Subjects and methods

Eight normal male volunteers, aged 23–31 years, taking no medication were recruited for the study. Detailed information on all aspects of the protocol was provided and written informed consent was obtained prior to the experiments. The study protocol was reviewed and approved by the ethics committee of the University of Cologne.

In a double-blind crossover study all subjects received an intravenous infusion of progesterone or vehicle in random order, separated by a minimum of 1 week. After at least 4 h of fasting the tests were performed in the metabolic unit starting at 13.30 h. At this time an indwelling cannula was inserted into antecubital veins
in each arm: one for the progesterone/vehicle infusion and the other for blood sampling. The participants remained in a recumbent position until the end of the study period (19.30 h).

**Day A**

At 14.00 h progesterone was administered as a continuous infusion over 315 min with a loading dose of 0.714 mg·kg⁻¹ body wt·h⁻¹ for 60 min, followed by a dose of 0.45 mg·kg⁻¹·h⁻¹ (Infusomat®, Braun, Melsungen, Germany). At 17.30 h (210 min after commencing progesterone) human CRH was given as a bolus injection in a dose of 1 µg/kg body wt (Corticobiss, Bissendorf, Hannover, Germany).

**Day B**

Vehicle instead of progesterone was infused. Otherwise, the protocol was identical to day A.

Progesterone was prepared according to the method of Bäckström et al. (16) with minor modifications: progesterone (Merck, Darmstadt, Germany) was dissolved in 75% ethanol (200 mg/10 ml) sterilized by filtration (0.22 µm, Millipore, Bedford, UK) and stored in ampoules. For vehicle infusion only ethanol (75%) was used (placebo). Immediately prior to the experiments progesterone (200 mg) or placebo was added to 490 ml of 5% glucose Ringer solution (Jonosteril Päd. III, Fresenius AG, Bad Homburg, Germany) containing 70 ml of human serum albumin solution (20%) (Serapharm, Münster, Germany).

Blood samples for determination of serum progesterone, cortisol and plasma ACTH were taken at 0 (start of infusion), 30, 60, 90, 120, 150, 180, 210 (CRH bolus injection), 225, 240, 255, 270, 285, 300 and 330 min. In addition, salivary samples for determination of free cortisol were collected.

Heart rate, blood pressure and temperature were recorded in frequent intervals throughout the study period.

Plasma ACTH was measured using a specific two-site immunoradiometric assay (Nichols Institute, Bad Nauheim, Germany). Serum cortisol (Incstar Corporation, Stillwater, MN) and serum progesterone (Diagnostic Products Corp., Los Angeles, CA), were determined by RIA using commercially available reagents. Salivary cortisol was measured as described previously (4, 17). All samples from an individual were analysed in a single assay.

Results are expressed as means ± SEM. Statistical significance was taken as p < 0.05. Analysis of hormone–time curves was performed by analysis of variance, taking repeated measures into consideration. The integrated areas under the hormone–time curves (0–330 min and 210–330 min, respectively) were calculated and compared using Student’s t-test for paired data.

![Figure 1](image_url)  
*Fig. 1. Serum progesterone concentrations during administration of high-dose progesterone (○) and placebo (●) in eight healthy males.*
Results

Progesterone was well tolerated and no side effects were noticed by the volunteers or the attending investigators. Heart rate, blood pressure and body temperature were not influenced significantly during the study period.

Within 30 min after commencing progesterone infusion the serum progesterone levels reached the target concentration of third trimester pregnancy (454 ± 31 nmol/l) and remained in the target range throughout the infusion period. After the end of the progesterone infusion a rapid fall in serum progesterone was observed (Fig. 1). Using steady-state progesterone concentrations from 210 to 300 min as baseline, a half-life of approximately 17.5 min for progesterone was calculated.

During vehicle administration the serum progesterone level remained in the normal range for healthy young males and demonstrated a small but significant increase after CRH (1.52 ± 0.23 nmol/l at 240 min vs 0.74 ± 0.14 nmol/l at 210 min; p < 0.01).

However, despite a highly elevated serum progesterone level for more than 3 h, the cortisol and the ACTH response to CRH remained unaffected compared to placebo. This held true also for salivary-free cortisol (Fig. 2). Similarly, baseline secretion of these hormones was not altered by progesterone infusion. Data for the areas under the curve are given in Table 1.

Discussion

Variable effects of progesterone on glucocorticoid action have been described. In vitro studies have found that progesterone can bind to glucocorticoid receptors (18–20) and diminish glucocorticoid action (9–12) in different systems. However, it has also been shown that progesterone can reduce the effect of hypothalamic extracts on in vitro ACTH secretion by rat corticotrophs (11, 13, 14), suggesting a weak glucocorticoid agonist action in the absence of corticosteroids.

The main finding of our study is the missing effect of acute high-dose progesterone administration on the pituitary–adrenal axis in healthy males. Thus, against our hypothesis (4) our investigation does not support the view that high unbound cortisol concentrations in late pregnancy are a result of the antiglucocorticoid action of progesterone (7). We have performed our study in males to reduce side effects due to the progestogen activity and to avoid influences of the menstrual cycle. Thus, we cannot fully exclude that females may exhibit antiglucocorticoid effects using a similar protocol. However, there is no experimental evidence for a sex-related difference in the activity of antiglucocorticoid steroids.

While some investigators found higher progesterone levels at delivery compared to the concentrations induced in our study (4, 21), others found progesterone concentrations similar to those in our volunteers (7, 22). Moreover, unbound cortisol increases from the 25th to 28th week onwards at a time when progesterone levels are lower than reached in our subjects. Thus, our findings cannot be explained by
missing the target progesterone concentrations. In addition, similar concentrations have been found to attenuate corticosteroid inhibition of \( \beta \)-endorphin release by rat anterior pituitaries in vitro (12).

Possibly a longer duration of infusion may be necessary to detect the antoglucocorticoid activity of progesterone. However, in vitro experiments (12) and in vivo studies in ewes (15) do not support this assumption. Infusion with the synthetic antiprogesterone 19-nor-steroid RU 486, a potent steroidal antoglucocorticoid compound, elevated ACTH and cortisol secretion in non-human primates within 60 min (23), indicating a rapid onset of antoglucocorticoid action. Using RU 486, it has been found that antoglucocorticoid activity may be evaluated more easily during the early morning hours (24). While this may be true for baseline cortisol secretion, increased ACTH and cortisol responses to CRH could be expected in the presence of an antoglucocorticoid, because a rapid effect of RU 486 on CRH-induced ACTH release has been demonstrated in the afternoon by Laue et al. (25).

Another explanation for the missing effect of progesterone could be the relatively low serum cortisol concentration in our volunteers, because the presence of long-term high cortisol levels, as in late pregnancy, may be required to detect the glucocorticoid antagonistic activity of progesterone (15). While this possibility cannot be fully excluded it seems to be unlikely, because serum cortisol was relatively high at the start of the infusion (possibly stress-induced) and even 120 min after CRH stimulation with high cortisol levels no difference became apparent.

Interestingly, progesterone induced no rapid or delayed increase in unbound cortisol, although it reached concentrations similar to total cortisol. Both steroids have been reported to compete for transcortin binding sites (26, 27). In males, transcortin concentrations are expected to be much lower than in third trimester pregnancy. Thus, our results question the ability of progesterone to alter efficiently the cortisol binding to transcortin in vivo.

In conclusion, by using short-term administration of high-dose progesterone in males, our study does not support the concept that progesterone concentrations in the range of third trimester pregnancy levels explain the refractory state to glucocorticoids in late pregnancy.

### References


### Table 1. Areas under the curve for 0–330 and 210–330 min during progesterone and vehicle infusion.

<table>
<thead>
<tr>
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<th>0–330 min</th>
<th></th>
<th>210–330 min</th>
<th></th>
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</thead>
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<tr>
<td>Progesterone</td>
<td>3.5 ± 0.4</td>
<td>5.7 ± 0.8</td>
<td>6.3 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>3.6 ± 0.3</td>
<td>414 ± 23</td>
<td>447 ± 24</td>
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<tr>
<td>Serum cortisol</td>
<td>278 ± 12</td>
<td>283 ± 9</td>
<td></td>
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<tr>
<td>Salivary cortisol</td>
<td>14.7 ± 1.9</td>
<td>14.2 ± 2.0</td>
<td>21.3 ± 2.7</td>
<td>20.3 ± 2.5</td>
</tr>
</tbody>
</table>

*No significant effect of progesterone was found.*
22. Scott EM, McGarrigle HHG, Lachelin GCL. The increase in plasma and saliva cortisol levels in pregnancy is not due to the increase in corticosteroid-binding globulin levels. J Clin Endocrinol Metab 1990;71:639–44

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