GLUCOCORTICOID EXCESS AND FETAL DEVELOPMENT IN WHITE WISTAR RATS

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Summary
Despite the clear physiological importance of endogenous glucocorticoids during development, there is a multitude of data to show that exposure to excess exogenous glucocorticoids during pregnancy correlates with reduced birth weight and adverse outcomes in the offspring, especially if glucocorticoids are administered during late gestation when growth is accelerating and presumably thus most susceptible to the catabolic effects of. As resulted from our preliminary experiments a short-term epicutaneous treatment with Fluocinolon-acetonid N in various age groups of rats induces structural and ultrastructural modifications of some organs accompanied by several biochemical disorders. In recent studies elsewhere we have reported that short-term and long-term epicutaneous applications of halogenated glucocorticosteroids in pregnant rats induced changes of thymus oxidative status of dams and newborn animals. Starting from the above observations we investigated the relationship between fetal brain development and fetal body weight evolution under glucocorticoid excess, induced by Fluocinolon N ointment, administered to female rats during gestation. Our results indicate that glucocorticoid excess in prenatal life modifies fetus development and might contribute to metabolic disorders in newborn rats reflected by decrease of body weight.

Keywords: Fluocinolon-acetonid N, fetal development

Introduction
Glucocorticoids (GC; cortisol or corticosterone) affect virtually all organ systems regulating metabolism, hydromineral balance, growth, development and neuronal function. Owing to their anti-inflammatory and immunosuppressive properties, GC have considerable clinical value, being one of the largest classes of marketed pharmaceuticals. They are currently among the most effective and widely used medications to treat chronic and acute inflammation (Rebeyrol et al 2012).

In dermatology, GC are the most widely used therapy. The introduction of topical hydrocortisone in the early 1950s represented a great advance over previously available therapies, but it was the first of the halogenated GC, triamcinolone acetonide, which started a revolution, cumulating in the appearance of the very potent agents available now. The enthusiasm for these highly effective agents was at its peak during the 1960s and 1970s, and perhaps inevitably, the more potent GC were often used inappropriately and indiscriminately.

Duration, dosage, and dosing regime and choice of the appropriate GC (a classification has been established based on their potency) and its mode of application depend on the clinical situation and take account of the risk/benefit ratio. These factors, together with an individual susceptibility of unknown reason, determine the occurrence and severity of the adverse effects. Overall, it can be stated that prolonged application is a high-risk factor, whereas total dose is of secondary importance. Side effects are usually more
severe after systemic than after topical application. Even topical therapy, however, can induce not only local, but also systemic adverse effects, as observed after cutaneous therapy for inflammatory dermatoses (Kis and Crăciun, 2006; Vegiopoulus and Herzig, 2007). The side effects occur with different prevalence, in different organs, and after different durations of therapy.

In pregnancies it is difficult to determine the impact of glucocorticoid treatment on cognition and adult behaviour in the offspring because these studies are confounded by subjects often born prematurely and so already at risk of delayed neurological development. Repeated prenatal betamethasone treatment did not induce alterations in toddler temperament, however a longer duration of exposure was associated with higher impulsivity scores in two-year-old children (Pesonen et al., 2009).

Starting from the above observations, we can hypothesise that abnormal hormone levels can modify the program of fetus development, causing changes which lead to dysfunction later in life. The mechanism of glucocorticoid excess induced tissue damage has not yet been fully elucidated. In literature there are very few data about glucocorticoid excess and its relationship to embryological development. Therefore, the main objective of this study is to determine correlations between fetal development and the variation of body weight of newborn rats under condition of glucocorticoid treatment.

**Material and methods**

The experiments were carried out in pregnant Wistar rats. The animals were kept under standardized bioclimatic conditions and fed on common rat chow, with water *ad libitum*.

Commercial Fluocinolone-acetonid-N ointment containing 25 mg Fluocinolon-acetonid-N/100 g excipient, was applied topically to the skin at 2cm², for five consecutive days, by smearing 50 mg ointment/100 g b.w on the inguinal region, the daily dose of ointment being equal to 12.5 µg/100 g b.w.

The pregnant animals were divided into the following groups:

- C-control group, pregnant, untreated animals.
- FC₁-treated group of pregnant animals, pregnancy of 9th-13th days Fluocinolon-N-treated animals
- FC₂-treated group of pregnant animals, pregnancy of 16th - 20th days Fluocinolon-N-treated animals
- FC₃-treated group of pregnant animals, pregnancy of 9th-20th days Fluocinolon-N-treated animals

After 16 hours of fasting and 24 hours following the cessation of treatments, the treated animals together with controls were sacrificed by exsanguinations. The body weights of newborn were measured with an accuracy of 0.00001 g immediately after excision.

**Results and discussion**

The body weight and viability of newborn rats. The parturition occurred on the twenty-first day of pregnancy. The autopsy was performed 24 hours after birth, but not all infants survived the critical first 24 hours. At necropsy we found in mothers FC₂ and FC₃ womb but dead fetuses. Therefore we considered necessary to distinguish between fetuses, neonates and viable infants number (Table 1).

### Table 1. Relative number (RNr.) of fetuses (F), newborns (Nb) and viable infants (Vi) after 24 hours of parturition, M (mothers)

<table>
<thead>
<tr>
<th>RNr</th>
<th>C</th>
<th>FC₁</th>
<th>FC₂</th>
<th>FC₃</th>
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<tbody>
<tr>
<td>F/M</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>86/7</td>
<td>12.28/100%</td>
<td>22/3/100%</td>
<td>50/6/100%</td>
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<tr>
<td>Nb/M</td>
<td>86/7</td>
<td>12.28/100%</td>
<td>22/3/100%</td>
<td>40/6/79.95%</td>
</tr>
<tr>
<td>Vi/M</td>
<td>84/7</td>
<td>12/97.71%</td>
<td>21/3/95.4%</td>
<td>33/6/5.5</td>
</tr>
</tbody>
</table>

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The numbers of fetuses and newborns in the control and in the early stage of pregnancy treated group are equal, the mortality in these groups was 0%. In the first 24 hours after parturition mortality is extremely low in these groups: 2.3% in control group, 4.5% in the FC\textsubscript{1}. In the late stage of pregnancy treated group FC\textsubscript{2}, intrauterine death has been high, 20.05% and 84.4% in the long-term treated group. In the first 24 hours after parturition mortality of the neonates were also high in FC\textsubscript{2} 17.5% and in FC\textsubscript{3} 100% (table nr.2).

<table>
<thead>
<tr>
<th>Table 2. Mortality rate</th>
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<tr>
<td>Mortality rate %</td>
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<td>intrauterine</td>
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<td>24 h after parturition</td>
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The mortality rate increased in uterus, as after 24 hours of parturition, with the duration of glucocorticoid treatment. The mortality rate was higher when the treatment was applied in the late stage of pregnancy (FC\textsubscript{2}), contrary to the results of treatment applied in the early stages of pregnancy (FC\textsubscript{1}), the time of glucocorticoid administration was the same. We measured parameters only from newborns surviving the first 24 hours after parturition, therefore only three groups of neonates were studied. We have no data on FC\textsubscript{3} newborns because none of them survived 24 hours after parturition.

The body weight of offspring FC\textsubscript{1} was statistically equal to the control value (Fig.1). We interpreted this observation in two ways. One explanation was that fluocinolone treatment did not cause long-term weight loss in newborns if the treatment was applied in the first stage of pregnancy. Nine days after treatment end the newborn's weight returned to the control value. The second explanation was way overdose of pregnant females with glucocorticoids did not affect the fetus development. Probably in these stage the neurons did not develop de GR sensitive receptors.

In the late stages of pregnancy treated group (FC\textsubscript{2}) offspring body weight was significantly reduced compared with the control group. This means that glucocorticoid overdose of mothers in the late stage of pregnancy affected embriological development more than in FC\textsubscript{1}. We interpreted this observation also in two ways: the glucocorticoid treatment caused weight loss but there was no time to returne to the control value because they were sacrificed after 24 hours, or, the glucocorticoid treatment in the late stage of pregnancy determined irreversible modification in fetus development. In this stage, neurons develop more sensitive GR receptors which cause stimulation of adrenergic neurons from brainstem, dopaminergic neurons from hypothalamus, down regulated the secretory function of hipotalamo-hipophyseal axis. The magnitude and nature of long-term effects of glucocorticoids might depend on tissue specific differences in ontogenetic expression of the GR (Bakker et al 2001). Prenatal glucocorticoid treatment is often GR specific, prenatal stress involves both MR and GR signalling and catecholamine release. And there is evidence that endogenous maternal and fetal glucocorticoids (and possibly other stress-related hormones) reduce birth weight and
have implications for the developing fetal hypothalamic-pituitary-adrenal axis (Teghetoff et al., 2009; Harris and Seckl 2011).

The weight loss of newborns in conditions of glucocorticoid excess were in accordance with our previous experimental findings that glucocorticoid treatment overregulated the somatotrope, tiretrole cells ultrastructure and function (Kis and Crăciun, 2006).

Earlier studies with betametasone have also observed reductions in birth weight of children exposed to prenatal corticosteroid treatment (Pesonen et al., 2009). The hypothalamic-pituitary-adrenocortical axis plays an important role in human behavior regulation and thus may have a role in explaining the associations of longer duration of betamethasone exposure with impulsivity and slower fetal growth with lower effortful control and higher negative affectivity (Seckl, 2008).

Conclusions
1. Prenatal corticosteroid treatment determines increasing of mortality rate in dose dependent manner.
2. Exogenous maternal and fetal glucocorticoids reduce birth weight.
3. Fluocinolone treatment in the late stage of pregnancy determined irreversible modification in fetus development.

References