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Research report

# Application of an antiandrogen during pregnancy infantilizes the male offsprings' behaviour

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**Abstract**

The present study was conducted to test the hypothesis that an application of an antiandrogen during pregnancy causes an infantilization of the male offsprings' behaviour later in life. The subjects studied were male guinea pigs whose mothers were either treated with an antiandrogen (flutamide and carrier) or a placebo (carrier only) during pregnancy. The mothers lived in groups of five females and one male. Application of the antiandrogen or the placebo took place on days 30, 32, 34, and 36 of pregnancy, the sensitive phase of foetal CNS sexual differentiation in guinea pigs. After weaning three groups of sons, whose mothers had received the antiandrogen (FT-sons) and five groups of sons, whose mothers had received the placebo (PT-sons) were established. Each group consisted of two males. From their 20th through their 100th day of age, the spontaneous behaviour of the males was recorded in their home cages in 5-day intervals. Additionally, blood samples were collected to determine serum cortisol concentrations. FT-sons and PT-sons did not differ in serum cortisol concentrations. However, distinct differences in behaviour occurred: FT-sons rested significantly longer with bodily contact than PT-sons. Additionally, FT-sons displayed more play-behaviour than PT-sons. These results point to a behavioural infantilization in males prenatally treated with antiandrogen. The behavioural differences between FT- and PT-sons are in accordance with previous studies in which a decrease of serum androgen concentrations in pregnant females living in an unstable social environment [Psychoneuroendocrinology 2001;26:503] and an infantilization of their sons' behaviour was described [Psychoneuroendocrinology 2003;28:67]. Thus, our study supports the hypothesis, that the decrease of androgen concentrations during pregnancy, caused by an unstable social environment, is responsible for the infantilization of the male offsprings' behaviour.


*Keywords:* Androgen; Antiandrogen; Development; Flutamide; Guinea pig; Infantilization; Prenatal environment; Prenatal stress

## 1. Introduction

The development of the central nervous system (CNS) is in part controlled by the endocrine system. Evidence suggests that sex steroids may cause permanent structural differences during brain development (so called: organizational effects) in addition to modulatory, activational effects in adulthood [21,26,38]. There are sensitive phases of brain development during which neurons are very responsive to androgens and estrogens [6,18,33,34]. A male-typical development of the CNS distinct from that of females is modulated by androgens [3,7,19,35]. The masculine CNS differentiation includes suppression of the behavioural and neuroendocrine patterns characteristic of the females, called "defeminization" and enhancement of the patterns characteristic of the males, called "masculinization". In the absence of sufficient androgenic stimulation the nervous system morphology takes a female course [5,19,21,32].

Because the pregnant animal's internal environment constitutes much of the embryo's external environment, it is reasonable to conjecture that major disturbances of the former may deteriously affect foetal development. This hypothesis is confirmed by studies on maternal stress during pregnancy, known also as prenatal stress. The influence of prenatal stress on the later expression of behaviour

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was first shown by Ward [32]. She described in male rats born by females stressed during pregnancy a decrement in maletypical and an increment in femaletypical sexual behaviour; the animals were demasculinized and feminized. Since that time many authors have shown effects of prenatal stress on the offspring (e.g., [8,23,33,39]). Most animal studies have been conducted in rodents [29]. In the majority pregnant females were subjected to nonsocial stressors. The most commonly used paradigms have been immobilization, intense illumination, heat or electric footshocks [32,34,37].

Our recent experimental work focussed on the influence of the prenatal social environment on guinea pig offspring. We have shown that social factors like the stability of the social environment during pregnancy have important effects on the offsprings' behaviour, endocrine systems, autonomic- and neuroendocrine functions: sons whose mothers had lived in an unstable social environment (which is experimentally induced by changing the group composition regularly) showed a behavioural infantilization that corresponded with a delayed development of the hypothalamic–pituitary–adrenal (HPA) axis and a decreased activity of the sympathetic-adrenomedullary system [13].

Surprisingly, living in an unstable social environment did not influence the cortisol concentrations of the pregnant female guinea pigs. However, androgen concentrations were significantly affected: pregnant females living in an unstable social environment showed a decrease in serum concentrations of dehydroepiandrosterone (DHEA) and dehydroepiandrosterone-sulfate (DHEAS) compared to controls [14]. Later in life, sons of females who had lived in an unstable social environment during pregnancy showed decreased serum concentrations of DHEA and a downregulation of androgen receptors in the medial preoptic area [15]. We hypothesised that in an early stage of foetal development, when CNS sexual differentiation takes place and when the production of DHEA by the foetus itself is low, the lack of maternal DHEA may lead to a lack of foetal DHEA. As a consequence, a downregulation of androgen receptors would occur and result in the male offsprings' behavioural infantilization later in life. Thus, reduced androgen concentrations of pregnant female guinea pigs, due to their life in an unstable social environment, might be responsible for the behavioural infantilization of their sons [14].

If so, the application of an antiandrogen to pregnant females during the sensitive period of foetal CNS sexual differentiation should result in the same behavioural infantilization that Kaiser and Sachser [13] described for sons whose mothers had lived in an unstable social environment during pregnancy, because antiandrogens block androgen receptors and, thereby, mimic reduced androgen concentrations. In the present study, we tested this prediction and compared the behaviour of sons whose mothers were treated during pregnancy either with an antiandrogen or a placebo.

## 2. Materials and methods

### 2.1. Subjects and housing conditions

The guinea pigs (*Cavia aperea* f. *porcellus*) used were descendants of a heterogeneous short haired and multicoloured stock of 40 animals obtained from a breeder in 1975. The animals were bred by chance in closed breeding colonies (∼20–50 animals). Through natural markings all animals were known individually.

The animals were kept under standardized conditions (photoperiod: 07:00–19:00 h; temperature: 20 ± 2 °C; relative humidity: ca. 60%). Commercial guinea pig diet (Höveler 1070, Höveler, Langenfeld, Germany) and water were available ad libitum. The water was twice a week fortified with ascorbic acid. This diet was supplemented regularly with fruits and hay. The floors were covered with standard bedding material (wood shavings) and straw. Cage cleaning took place once a week. All experiments were announced to the competent local authority and were approved by the 'Tierschutzbeauftragter' of the University of Münster.

### 2.2. Housing conditions of the mothers

The sons were born by mothers living in groups of one male and five females. Eight such groups had been established in the following way: females were transferred from their natal colonies to 1 $m^2$ enclosures on their 20th day of age, which is the time around weaning, until five non-related females were present. The females were placed together with an adult male (6–12 months of age). The age difference between the females of the same group did not exceed 40 days.

At about 28 days of age the females were mated for the first time and gave birth to their first litter after about 68 days of gestation. Thereafter, they were mated postpartum and had their second gestation. After weaning (20 days of age) the offspring were taken out of the enclosures.

### 2.3. Antiandrogen treatment

20 females (4 groups with 5 females each) were treated with the antiandrogen flutamide (Sigma–Aldrich Chemie GmbH, Steinheim, Germany). The antiandrogen was injected intramuscularly into the thighs of the hind legs of the pregnant females (FT-females) at a dosage of 20 mg/kg body weight in 0.36 ml sesame-oil. The 20 females of the control groups (four groups with five females each; PT-females) were injected with sesame-oil alone. The injections took place on day 30, 32, 34 and 36 of the females second pregnancy (the sensitive phase of the foetal CNS sexual differentiation in guinea pigs is from the 30th to 37th day of pregnancy: [7,24]). The applications occurred always at 12:30 a.m. Flutamide is a strong antiandrogen that induces morphological feminization in male rats [11,12,21] and guinea pigs [31] when it is administered in high doses during fetal development. As noted in the Introduction, in our study the antiandrogen is used to