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# Ambient temperature and pregnancy influence cortisol levels in female guinea pigs and entail long-term effects on the stress response of their offspring

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ABSTRACT

Mammals generally respond to the important metabolic requirements imposed by thermoregulation and pregnancy by increasing plasma concentrations of glucocorticoid that promote the mobilization of body reserves and enhance energy use by tissues. This study examined the impact of distinct ambient temperatures and reproductive status on cortisol plasma levels in female guinea pigs (*Cavia aperea f. porcellus*). We also examined cortisol profiles of their offspring. Forty adult females were placed in individual boxes, 20 were exposed to a neutral thermal regime (mean ambient temperature 22.1 ± 1.5 °C) and 20 were maintained under a cool thermal regime (15.1 ± 1.5 °C). Within each treatment, 12 females were pregnant and 8 were non-pregnant. Pregnancy generated a marked elevation of baseline cortisol. Ambient temperature also affected cortisol concentrations. Compared to the pregnant females from the neutral thermal regime, pregnant females maintained under cool conditions exhibited lower baseline levels of cortisol, were less active, but they displayed a greater stress response (i.e. rapid increase of plasma cortisol) following handling. Thermal treatment did not influence reproductive output, reproductive effort, or offspring characteristics. This suggests that pregnant female guinea pigs cope with cool (but not extreme) thermal conditions by reducing activity and baseline cortisol levels, possibly to save energy via an adaptive response. Interestingly, the greater amplitude of the stress response of the cool regime females was also observed in their offspring 2 months after parturition, suggesting that hormonal ambience experienced by the individuals *in utero* shaped their stress response long after birth.



## 1. Introduction

Glucocorticoid hormones are notably involved in the mobilization and use of energy in various physiological processes, including cardiovascular, metabolic, immunologic and homeostatic processes. These hormones are therefore central in the energy budgets of vertebrates [2,8,11,14,16,80]. Hormonal regulations are complex however, and different factors such as stress, age, photoperiod, and social context influence plasma glucocorticoid levels [6–8,62]. In female mammals, thermoregulation and reproduction involve major energy expenditure [52]. As both of these processes are under hormonal regulation, including glucocorticoid hormones, interactions between ambient temperature, gestation, and glucorticoid levels are thus expected [61,62]. This issue remains poorly explored however. Glucocorticoids also play important roles in the stress response. The concept of stress is complex and subjected to various definitions (e.g. water balance stress, energetic stress, and emotional stress are all investigated in different ways and they involve different effectors). In a broad context however, individuals are considered as stressed when their neuro-physiological demands exceed their regulatory capacities [5]. Stress is thus generally perceived as a temporary state existing between homeostasis and pathology, and in most cases stressors provoke consistent physiological responses with a rapid elevation of the plasma levels of several circulating hormone such as catecholamine, glucocorticoid, and vasopressin notably [5,13]. In the current study we limit the investigations of stress to marked elevations of glucocorticoid plasma levels under different ambient temperatures (an environmental stressor), considering reproductive status (a physiological stressor), and following handling (thereby mimicking a predator attack).

The influence of ambient temperature on reproduction has been documented in mammals, mostly in livestock, perhaps owing to their economic value [18,25,50,70]. Heat-stress, for example, can perturb reproduction in mammals [26,35]. However, very few studies have been performed on the effect of cold ambient temperatures on pregnant females and their offspring [85]. Embryos are sensitive to intra-uterine environmental conditions; their development and thus the resulting neonate phenotypes are optimized under stable conditions [22]. Pregnant females are under strong selection to buffer the perturbations that may reach their embryos [20] and hormones play a central role in these buffering processes

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[84]. When pregnant females are exposed to low temperatures, strong hormonal responses are expected. The embryos may consequently be exposed to hormonal fluctuations that can in turn influence their phenotype. Steroids are especially active in this process as they cross biological membranes easily and thus rapidly reach the embryo [86]. A considerable proportion of maternal effects on offspring phenotypes are indeed mediated via hormones [55]. The possible cascade of effects of low ambient temperatures during gestation on offspring phenotypes mediated by hormones has not been examined in mammals.

The energetic impacts of gestation and cool ambient temperatures should be particularly severe in relatively small and precocial mammals: the production of large and well-developed neonates requires a considerable reproductive investment during gestation and this is cumulated with an unfavorable body mass/surface ratio for endothermy in small mammals [31]. Guinea pigs (*Cavia aperea f. porcellus*, Caviomorph rodents) provide an excellent opportunity to examine these issues. In this species pregnancy lasts 68 days on average, entails a heavy maternal investment and females give birth to very large and well-developed offspring relative to their own size [44]. On average, relative litter mass represents 30% of the post-parturient maternal mass. Maternal mass can increase by more than 50% during gestation; impeding maternal mobility and sustained by an intensive foraging activity. Neonates are almost independent and the lactation period is very brief [43,44]. Because neonates are physiologically mature for many functions (e.g. central nervous system, endocrine and cardiovascular systems), their phenotypes, including endocrine characteristics, should largely reflect the impact of the prolonged intra-uterine period. Adult females are relatively small (approximately 800 g), and thus are subject to the difficult physiological challenge of maintaining their body temperature under low thermal conditions. Adding to the suitability of this species for this study is the existing background data: the reproductive physiology and ecology of these animals have been intensively studied [40,44,71,72].

We experimentally examined the influence of distinct (neutral versus cool) ambient temperatures on baseline and stress cortisol levels in pregnant and non-pregnant female guinea pigs. We used in this study the cortisol level as a proxy of the stress response. Although we thus assessed only one aspect of an otherwise complex stress response, the use of changes of cortisol levels (routinely measured in vertebrates) has been validated and is now widely employed [13]. We also assessed the impact of experimental treatment on reproductive output and the offspring. We notably assessed baseline and stress cortisol levels of the offspring 2 months after birth. This issue is important to determine if environmental conditions can influence offspring stress physiology, a prerequisite to explore the adaptive value of maternal effects [9]. We addressed two main questions:

(1) Do gestational status and ambient temperature influence maternal cortisol levels?
(2) If so, does this translate into different stress responses in the offspring?

## 2. Materials and methods

### 2.1. Study species

The domestic guinea pig reproduces throughout the year and, in contrast to rats and mice, irrespective of photoperiod, assuming sufficient food and thermally neutral ambient conditions [71]. Caviomorphs are distinct from other rodents as they produce extremely precocial offspring, guinea pigs in particular. Compared to altricial species, neonates are physiologically mature (except for reproductive functions), agile and relatively independent at birth, resembling miniature adults: they have open eyes, exhibit fully developed fur, feeding apparatus, and start to forage almost immediately after birth. In guinea pigs, most of the offspring development is achieved before birth [44]. Neonates survive weaning at 5 days old [77], and in experimental conditions it is possible to separate them from their mother 6 days after parturition. Pregnancy entails a massive maternal investment and enhances feeding rate [44]. Maternal body mass increases markedly (50% elevation) during gestation, and impedes mobility due to the overload-handicap. Overall the potential effects of post-natal maternal care are very limited compared to the long gestation period during which most of the morphological characteristics are set.

Previous studies provided an important background on guinea pig life history traits, and reproductive physiology and ecology [4,36,38,47,59,65]. Notably, the role of the HPA (hypothalamic–pituitary–adrenal axis) in relation with social status and stress responses has been investigated [29,30,39,53]. Importantly, these studies shown that in guinea pigs plasma variations of glucocorticoids can be used as a proxy of the stress response. But they also revealed that such response is relatively independent of several confusing factors. For instance, social environment affects behavior and androgen levels, but not cortisol concentrations in pregnant females [37] and their young [41]. These results limited possible confusing social influence impacts during pregnancy on plasma cortisol levels, and thus enabled us to focus on the impact of ambient temperatures in interaction with reproductive status.

### 2.2. Experimental design

Forty adult female guinea pigs were involved in the experiments (February 2008 to April 2008). Individuals originated from a colony maintained at the Centre d'Etude Biologique de Chizé (France). Using natural color markings, each individual was easily identified (e.g. variations of the main color morphs: "yellow", "black", "brown", and "gray"). All the adult females involved had previously successfully produced 1 or 2 litters, but none of them had recently reproduced at the onset of the experiment. At least 100 days had elapsed between the last parturition and the beginning of the experiment.

We placed each female with an adult male (randomly selected from twenty males) in an individual cage for 10 days, and then with a different male for the same amount of time. We expected that most (i.e. >70%) of the females would become pregnant and that the rest of the females would not reproduce for various uncontrolled factors as observed in closely related experimental designs [71]. We then randomly allocated the females to two thermal treatments. Maternal body mass, body size, color morph and age did not differ significantly between the two experimental groups (Wilcoxon–Mann–Whiney-*U* test, all $p > 0.1$).

Most endotherms better tolerate cool rather than hot ambient temperatures; therefore we used a neutral regime versus cool regime design [28]. In guinea pigs, thermal conductance is relatively low and stable when individuals are placed under ambient temperatures (Ta) close to 20 °C; central body (colonic) temperature is easily maintained at 38 °C when Ta is between 22 °C and 30 °C [24]. For the current experiment, we set up a "cool" mean temperature regime at 15 °C, and a "neutral" mean temperature regime at 22 °C. Such experimental ambient temperatures, although distinct, were not particularly harsh for the animals (the 15 °C cool regime was well tolerated and no sign of disorder was observed), thereby limiting a potential overstress impact on reproduction. The experiment was performed in winter in a large shed that protected the animals from bad weather (extreme cold temperatures and precipitations) and buffered external temperature fluctuations. Each of the 40 females was kept in an individual box (60 cm × 50 cm × 35 cm) with a wood shelter (20 cm × 15 cm × 15 cm) and hay as substratum, a