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# The effect of isoflurane anaesthesia and vasectomy on circulating corticosterone and ACTH in BALB/c mice

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ABSTRACT

The use of blood corticosterone and faecal corticosterone metabolites as biomarkers of post-surgical stress and pain in laboratory animals has increased during the last decade. However, many aspects of their reliability in laboratory mice remain uninvestigated. This study investigated serum corticosterone and adrenocorticotropic hormone (ACTH) in mice subjected to isoflurane anaesthesia and vasectomy, and mice subjected to isoflurane anaesthesia without surgery. Serum levels of corticosterone and ACTH after pretreatment with dexamethasone were analysed to provide further information about the stress hormone profiles. Vasectomy resulted in an increase in corticosterone for at least four hours after surgery with a peak 30 min after the mice regained righting reflex. Mice subjected to isoflurane anaesthesia without surgery had the highest level of serum corticosterone 5 min after regained righting reflex and the level returned to baseline levels four hours after the procedure. In vasectomised mice, treated with dexamethasone, high levels of corticosterone remained 30 min after the procedure, whereas the anaesthetised mice, treated with dexamethasone, had significantly lower levels of corticosterone compared to anaesthetised mice not treated with dexamethasone. Thus, dexamethasone effectively inhibited the corticosterone response in the anaesthetised-only mice, but not in the mice subjected to surgery. In conclusion, both isoflurane anaesthesia and vasectomy during isoflurane anaesthesia resulted in an increase in serum glucocorticoids, but the negative feedback mechanism of newly operated mice, was altered. This may have consequences for the interpretation of glucocorticoids measurements as a biomarker of post-surgical stress in mice.



## 1. Introduction

Pain and stress may be significant confounders in various research settings and animal models [14,61,74,83]. Furthermore, letting animals suffer represents a serious ethical problem, and one should strive towards eliminating all unnecessary pain and stress from animal experimentation. This, however, requires a thorough knowledge about the extent to which various standard procedures cause pain and stress in the animals. Due to the increased use of genetically modified mice, vasectomy is a common surgical procedure in laboratory animal facilities. The traditional procedure of vasectomy involves tissue injury and interference of the abdominal cavity and has been shown to result in behavioural changes indicative of pain [18,33,86]. Furthermore, the procedure requires general anaesthesia, which may be a significant stressor for the animals [78]. Optimal pain and stress assessment and alleviation may therefore significantly improve the welfare of the animals subjected to this procedure. However, in mice, it is not fully clear to what extent the procedure affects the hypothalamic–pituitary–adrenal (HPA)-axis and corticosterone levels in blood and faeces.

Corticosterone is a main effector hormone in the stress response of mice and an important mediator of the animal's survival response [12]. It may, however, affect the fitness of the animal, since both acute and chronically elevated glucocorticoid levels are associated with immunological dysfunction, metabolic and reproductive impairments, behavioural changes and other negative health implications for the subject [22,56,62]. In relation to surgical procedures, glucocorticoids are released in response to the anaesthesia, the tissue injury, and the post-surgical nociceptive signalling (for review see [17]). In humans, the emotional stress of going though surgery may also significantly affect the physiological stress response [51]. However, the relation between stress and pain is complex, and stress may both attenuate [8,47,48,83] and enhance the pain reaction [15,39,84] depending on the stressor [63].

Glucocorticoid levels in serum or plasma, urine and faeces have been extensively used as biomarkers of stress and indirectly of pain in many species [25,29,41,60,65,70]. Measuring faecal glucocorticoid metabolites (FCM) has several advantages compared to quantifying circulating glucocorticoid levels, when assessing the animal's HPA-axis response. Whereas blood levels of corticosterone may provide a snapshot of the stress level, faecal corticosterone and

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corticosterone metabolites represent a true integrated amount of the steroids released over time. Furthermore, quantification of FCM is less likely to be biased by the sampling technique, since the sampling technique does not involve interaction with the animal during the period of interest. However, the correlation between pain related to surgery and FCM levels has not yet been proven conclusively in mice. Wright-Williams and colleagues evaluated FCM levels, obtained at different time points following surgery by stroking the tail of the mouse and applying light pressure to the back to encourage the mouse to defecate. In their setup, vasectomy and isoflurane anaesthesia resulted in elevated FCM levels eight hours post-surgery [86]. By measuring FCM levels over 24 h, we have however not been able to detect any increase in FCM the day after vasectomy and isoflurane anaesthesia, even though other parameters indicated a pain reaction [33]. This agrees with a study by Adamson and colleagues, in which no increase in FCM was detected at 12 and 24 h after surgical removal of the mammary fat pad in female mice anaesthetized with sodium pentobarbital, even though food consumption and wheel running activity indicated presence of pain [1]. This could reflect that post-surgical pain is mainly an acute response with highest pain intensity hours after the procedure [64]. However, a recent study by Matsumiya et al. indicated that pain after laparotomy may last for 36 to 48 h [53]. Furthermore, serum levels of corticosterone in response to permanent catheterization and isoflurane anaesthesia have shown that the surgical procedure activated the HPA-axis, and that the rise in serum corticosterone could be attenuated with buprenorphine. However, the increase seen in serum corticosterone could not be detected in faeces 24 h after the procedure [72]. Furthermore, serum levels of corticosterone may not always accurately reflect post-surgical pain in mice. In a recent work by Tubbs et al., no difference in serum corticosterone could be detected between mice subjected to partial hepatectomy and isoflurane anaesthesia receiving different analgesic strategies, despite other parameters indicating that not all treatments provided effective pain relief [75].These results are surprising, as several studies have demonstrated a good correlation between stimulation of the HPA-axis and faecal glucocorticoid levels in rats [35,42,69], companion and farm animals [10,29,54,71], poultry [11], non-human primates [59,82] and several wild life species [81]. However, due to pronounced interspecies differences in both anatomical and biochemical parameters [3,57,58,73] in relation to stress or pain, it is not possible to draw analogous conclusions from other species to the specific situation present in the mouse. Many aspects of using stress hormones, both in faeces and in blood, as indicators of post-operative pain and stress therefore still need to be investigated.

The aim of the present study was to evaluate the stress response seen after abdominal vasectomy by measuring serum corticosterone and ACTH at various time points after the procedure, with or without dexamethasone pre-treatment. Dexamethasone is a potent suppressor of the endogenous HPA-axis through negative feedback mechanism on the pituitary gland [28,67]. The results were compared to a group subjected to the same handling and isoflurane anaesthesia as the vasectomised mice, but not subjected to surgery. As previous studies [1,33] have shown that FCM levels may be unreliable as an indicator of stress in newly operated mice, since it does not increase in faeces as expected, it was hypothesised that corticosterone might be re-circulating in the blood stream due to post-surgical ileus in the vasectomised mice. This would result in corticosterone maintaining high levels in the blood for a longer duration, compared to the actual amount initially released. This would possibly affect the negative feedback mechanism of the HPA-axis. Thus, it was expected that dexamethasone pre-treatment would affect the corticosterone and ACTH profile differently depending on whether or not the animals underwent surgery.

## 2. Materials and methods

The animal experiments performed in this study were approved by the Animal Experiments Inspectorate under the Danish Ministry of Justice (license number 2009/561-1687). All procedures were performed in accordance with the *Guide for the Care and Use of Laboratory Animals* [32] in a fully AAALAC accredited facility.

### 2.1. Animals and housing conditions

A total of 85 male BALB/c mice, aged 8–10 weeks, weighing 25.7 ± 1.3 g (mean ± SD) were obtained from Taconic, Ry, Denmark. The mice were housed individually after the surgery to ensure a calm post-surgical recovery period. Short term single housing of mice is generally not considered stressful [7,27], and the mice were housed individually one week prior to the study for habituation. The mice were housed in Universal Euro II Type Long disposable cages (Innovive Inc., San Diego, USA). The cages are made of 100% PET, BPA-FREE with outside dimensions: 37.3 cm L × 23.4 cm W × 14 cm H. Food pellets (Altromin 1319, Brogaarden, Gentofte, Denmark) and acidified tap water were provided *ad libitum*. Wooden chips (Tapvei Oy., Kortteinen, Finland) were used as bedding. Bite bricks (Tapvei®, Kortteinen, Finland), Enviro-dri® nesting material (Shepherd Specialty papers, Watertown, TN, USA) and a cardboard roll (Mini fun tubes, Lillico, Brogaarden, Gentofte, Denmark) were provided as environmental enrichment. Room temperature was maintained at 20 ± 2 °C, air humidity was 55% ± 10% and the air was changed approximately 50 times per hour. The light was regulated with a 12/12 h dark/artificial light cycle with the lights on at 6:30 am.

### 2.2. Study design

The mice were randomly divided into six groups (Table 1); the mice of group 1 (ANAEST) were subjected to isoflurane anaesthesia for 15–20 min and the mice of group 2 (SURG) were subjected to isoflurane anaesthesia and vasectomy (mean duration 15–20 min). Group 3 (CTRL) were left undisturbed until blood sampling and served as control animals. Mice in groups 4, 5 and 6 were treated with 3.0 mg/kg dexamethasone subcutaneously and subjected to either 15–20 min isoflurane anaesthesia without surgery (ANAEST + DEX), isoflurane anaesthesia and vasectomy (SURG + -DEX), or no procedure (CTRL + DEX) and blood was sampled 45–50 after the dexamethasone injection. A sample size estimate based on expected ACTH values with a power of 80%, an alpha level of 0.05 and a 3 to 4-fold increase estimated as biological relevant, estimated sample sizes of 5 animals per group. As we expected the variance to decrease at the end of the study period, fewer animals were used at e.g. the 24 h time point. A blood sample was obtained from each mouse at various time points as described in sampling methods below. Each mouse was subjected to only one procedure and sampled at only one time point. In contrast to the standard procedure of vasectomy in the facility, no analgesia was provided to any animal in the present study. Analgesic treatment with common used opioids, NSAIDs or local analgesia may besides reducing pain sensitivity and post-operative pain behaviour, affect the glucocorticoids level as demonstrated in various species and experimental setups [19,26,49,71,72,86]. Analgesia was therefore withheld as it would likely interfere with the pain and stress reaction in mice subjected to surgery. The lack of analgesia in the present study was thus necessary, in order to gain unbiased information on how the corticosteroid response is regulated after a surgical procedure, with the associated surgical stress response. However, as animal suffering from pain is of serious welfare concern, humane endpoints were established prior to the study,