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Blood plasma collected after adrenocorticotropic hormone administration during the preovulatory period in the sow negatively affects *in vitro* fertilization by disturbing spermatozoa function

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ABSTRACT

Successful fertilization is essential for reproduction and might be negatively affected by stressful events, which could alter the environment where fertilization occurs. The aim of the study was to determine whether an altered hormonal profile in blood plasma caused by adrenocorticotropic hormone (ACTH) administration could affect *in vitro* fertilization in the pig model. In experiment 1, gametes were exposed for 24 hours to plasma from ACTH-treated, non-ACTH–treated sows, or medium with BSA. Fertilization, cleavage, and blastocyst rates were lower in the ACTH group compared with the no ACTH or BSA control groups ($P < 0.01$). In experiment 2, the exposure of matured oocytes for 1 hour before fertilization to the same treatments did not have an impact on their ability to undergo fertilization or on embryo development. In experiment 3, spermatozoa were incubated for 0, 1, 4, and 24 hours under the same conditions. There was no effect of treatment on sperm viability. The percentage of acrosome-reacted spermatozoa remained higher in the ACTH group compared with the non-ACTH–treated group through the incubation period ($P < 0.001$). Protein tyrosine phosphorylation (PTP) patterns were also affected by treatment ($P < 0.001$). The presence of an atypical PTP pattern was higher in the ACTH group at all the analyzed time points compared with the BSA and no ACTH groups ($P < 0.001$). In conclusion, this altered environment may not affect oocyte competence but might affect the sperm fertilizing ability through alterations in the acrosome reaction and correct sequence of PTP patterns.

1. Introduction

Stress does not necessarily cause infertility, but because stressful events can interfere with the hypothalamic-pituitary-adrenogonadal axis, such a delicate process as reproduction may be affected. In stressful events, the hypothalamus releases corticotropic-releasing hormone, which triggers the release of adrenocorticotropic hormone (ACTH) from the pituitary and eventually cortisol from the adrenal cortex [1]. It is well known that the activation of the hypothalamic-pituitary-adrenal axis may interfere with reproductive efficiency mainly through the alteration in gonadotropin secretion [2]. Because of the complex nature of the neuroendocrine system, pathways linking psychological factors with alterations in reproduction are likely to be multifactorial. Available evidence is still limited, but there is a plethora of information suggesting negative

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effects of stress on fertility. In this regard, maternal psychosocial stress and infertility have been linked in humans [3,4]. Stress also reduced the probability of conception during the fertile window in women [5]. In addition, success rates for IVF or gamete intrafallopian transfer were affected by stress [6]. In the latter study, the number of oocytes collected and fertilized, as well as pregnancy rates and live birth delivery, were negatively influenced by stress. Moreover, natural occurring stressors unrelated to infertility problems may reduce the outcome of IVF by impacting the number of retrieved oocytes [7]. In animal models such as the mouse, psychological stress coincident with follicular growth and oocyte maturation negatively affects the oocyte competence to develop to the blastocyst stage [8,9], but very little is known about the underlying mechanisms that may directly impact oocyte competence after psychogenic stress. Recently, it has been shown that maternal restraint increased aneuploidy during mouse oocyte maturation both *in vivo* and *in vitro*, impeding metaphase I assembly by inhibiting mitogen-activated protein kinase activity [10]. Additionally, maternal restraint accelerated the progression of anaphase I and concomitantly downregulated the spindle assembly checkpoint [10]. To our knowledge, there are no studies evaluating the effect of psychological stress specifically during the fertilization period. An altered environment at the fertilization site might have a negative impact on gametes function leading eventually to a failure in fertilization.

The pig is a social species, sensitive to psychosocial stress that may have an impact on reproduction. Additionally, the influence of stress on reproductive function has been extensively studied in the sow in relation to the stage of the estrous cycle [11]. Simulating psychological stress for approximately 48 hours from the onset of estrus (by repeated exogenous ACTH administration) disturbed the duration of reproductive behavior and changed the hormonal profile in sows [12]. Moreover, ACTH administration caused a loss of either oocytes or embryos and possibly also increased the speed of transportation of oocytes-embryos through the oviduct [13]. However, it was not possible to address the potential influence of simulated stress mimicked by ACTH administration directly at the gamete level in the aforementioned *in vivo* studies. We have recently determined that an altered hormonal environment provided by a brief exposure to plasma from ACTH-treated sows during *in vitro* oocyte maturation could induce alterations in actin cytoskeleton and mitochondrial patterns in oocytes, but these changes might not hamper the subsequent *in vitro* embryo development [14]. However, the likely impact of an altered hormonal environment derived from ACTH administration during fertilization has not been analyzed yet. Evaluating the effects of exposure to an abnormal endocrine environment would help to elucidate possible processes affected by psychological stress at the oocyte-spermatozoon level.

Thus, the general aim of the present study was to investigate whether fertilization is affected by an altered hormonal environment derived from ACTH administration. To address this issue, blood plasma collected at ovulation from sows that had experienced simulated stress through repeated ACTH administration during the periovulatory period was added during IVF. The plasma was partially characterized, because cortisol and reproductive hormone concentrations were previously assayed and the collection time in relation to ovulation was also known [12]. To provide more exhaustive information about the effects observed in the first part of the study, additional experiments were performed. For these experiments, the same plasma collected at ovulation was added separately to both the female and male gametes to specifically evaluate (1) whether the oocyte exposure to an altered fertilization environment has an impact on subsequent fertilization and embryo development, and (2) if this altered hormonal profile caused by ACTH administration might induce changes in sperm function.

2. Materials and methods

Unless otherwise stated, all the reagents used were purchased from Sigma-Aldrich, Stockholm, Sweden.

2.1. Plasma samples and animals

The plasma samples used in this study were collected through jugular vein catheters from sows that were either subjected to ACTH administration (n = 3) or control animals (n = 3) from a previous experiment [12]. Therefore, the present study was carried out without performing any additional *in vivo* experiments to collect blood plasma samples. Briefly, synthetic ACTH (5 μg/kg bodyweight) or 0.9% NaCl was administered to the sows every 4 hours from the onset of standing estrus (∼48 hours before ovulation) until ∼12 hours after ovulation. Ovulation was monitored by transrectal ultrasonography. Plasma collected at ovulation (±2 hours) from each group was pooled for culture supplementation during IVF (see subsequently). The mean plasma levels of reproductive hormones and cortisol are shown (Table 1). Boars (n = 2) were kept on straw in individual pens at the Division of Reproduction, Department of Clinical Sciences (SLU), Uppsala (Sweden). Water was provided *ad libitum* and they were fed according to Swedish standards [15]. The Ethics Committee for Experimentation

Table 1
Mean (±SD) hormonal concentration in pooled plasma collected from ACTH-treated or control sows at ovulation (±2 hours).

Group	Cortisol (nmol/L)	Progesterone (nmol/L)	LH (μg/L)	17-β-Oestradiol (pmol/L)	Inhibin alpha (ng/mL)
Control (n = 3)	97.4 ± 44.6	1.9 ± 0.3	0.4 ± 0.4	3.3 ± 0.6	0.3 ± 0.03
ACTH (n = 3)	566.4 ± 73.3	6.9 ± 3.5	0.8 ± 0.5	6.7 ± 1.2	0.3 ± 0.1

Adrenocorticotropic hormone was administered every 4 hours during the preovulatory period, from the beginning of estrus (∼48 hours before ovulation) until ∼12 hours after ovulation. Monitoring of ovulation was performed by transrectal ultrasonography. Adapted from Ref. [12].
Abbreviation: ACTH, adrenocorticotropic hormone.