Repeated Acute Activation of the Hypothalamo-Pituitary Adrenal Axis Prior to and during Estrus Did Not Affect Reproductive Performance in Gilts

A.I. Turner, P.H. Hemsworth, P.E. Hughes, and A.J. Tilbrook

ABSTRACT

We investigated the effects of repeated acute activation of the hypothalamo-pituitary adrenal axis, prior to and during estrus, on reproduction in gilts. Individual gilts (n = 24 per treatment) either served as controls or were subjected to daily acute stress ("negative handling," brief electric shock with a battery-operated prodder during confinement with the experimenter) commencing, on average, 8 days prior to estrus. Gilts subjected to negative handling had a significant elevation in plasma concentrations of cortisol that lasted at least 3-4 h, and these gilts were slower than control gilts to approach and interact with the experimenter in a standard test. Nevertheless, reproductive performance—as measured by sexual receptivity and proceptivity, ovulation, the percentage of gilts that became pregnant, the number of embryos 20-21 days after insemination, and the weight of embryos—was not affected by repeated acute activation of the hypothalamo-pituitary adrenal axis. Our results suggest that repeated acute activation of the hypothalamo-pituitary adrenal axis prior to and during estrus does not affect the factors that control estrus and ovulation in gilts.

INTRODUCTION

A number of studies have shown that prolonged activation of the hypothalamo-pituitary adrenal axis can impair reproduction in female pigs. For instance, some aspects of reproduction have been impaired by long-term handling with a battery-operated stock-moving prodder [1], long periods of overcrowding [2], and housing in tether stalls [3]. Furthermore, prolonged elevation of circulating cortisol due to administration of ACTH [4-9] or cortisol [4, 8, 9] to female pigs or of ACTH, cortisol, or a "stress" treatment to male pigs [10] has been found to impair reproduction. It has been proposed that females may be particularly susceptible to the effects of short-term stress during the series of endocrine events that lead up to estrus and ovulation [11]. Moberg [11] proposed that disruption of this series of endocrine events is likely to jeopardize reproductive performance in females. This proposal has never been thoroughly tested, although there is limited evidence from a number of studies to suggest that short-term stressors during this period may disrupt reproduction in females. Various aspects of reproduction or reproductive endocrinology have been impaired in sheep by laparoscopies [12], shearing [13, 14], confinement [15], and "wetting-stress" [16]; in cows by use of a cattle prodder during confinement [17]; in monkeys by restraint [18]; and in rats by immobilization [19]. In pigs, the stress of surgery during the follicular phase of the estrous cycle was shown to delay the onset of estrus in some but not all gilts [20]. In the intensive production of pigs, a number of routine management practices result in an elevation of cortisol in the females (e.g., electrical stimulation [21], use of a snout rope [22, 23], mixing of unfamiliar pigs or relocation [24], transportation [25], and detection of estrus by introduction to a boar in his pen [26, 27]). Therefore, it might be expected that reproductive performance would be impaired if these practices were used during the period leading up to estrus and ovulation.

Recently, we investigated the impact on reproduction of intense courtship of gilts by boars for 13 days prior to estrus [27]. There was a significant transient elevation of cortisol in gilts following 5 min of intense courtship by a boar in his pen for the detection of estrus. In contrast, there was not a significant elevation of cortisol when gilts encountered fence-line exposure to boars, in which courtship was limited and estrus was checked by the experimenter's applying pressure to the back of the gilt. Despite the differences in stimulation of cortisol secretion between these treatments, reproduction was not impaired, which implies that repeated activation of the hypothalamo-pituitary adrenal axis prior to estrus might not disrupt reproduction. Nevertheless, these results are not conclusive because the hypothalamo-pituitary adrenal axis might not have been sufficiently activated and/or because sexual stimuli from the boar may have counteracted any negative effects of increased secretion of cortisol [27]. Therefore, to investigate whether repeated activation of the hypothalamo-pituitary adrenal axis can impair reproduction, we used a handling treatment that is known to elicit a substantial release of cortisol [1] and that does not involve stimuli from boars. This treatment involved confinement of each gilt with the experimenter, who regularly approached and shocked the gilt using a battery-operated prodder. When a similar treatment was imposed on pigs regularly during rearing, it was shown to result in a significant elevation of plasma concentrations of cortisol, in fewer pigs approaching and interacting with the experimenter in a standard test, in a reduced proportion of gilts that became pregnant after mating at second estrus [1], and in a reduction in growth rate of young pigs [28-30]. This "negative handling" treatment is a good model for the study of acute stress as it reliably induces activation of the hypothalamo-pituitary adrenal axis and is known, after prolonged use, to result in compensation of various physiological parameters such as growth. We used this negative handling procedure to test the hypothesis that repeated acute activation of the hypothalamo-pituitary adrenal axis during the period before estrus and during estrus will impair reproductive performance in gilts.

MATERIALS AND METHODS

Animals

Puberty was induced in 48 gilts (Large White x Landrace; 194-218 days old) by regular fence-line exposure to
boars for 3 wk. Exposure of gilts to boars occurred in an
arena that was adjoined on two sides by stalls containing
mature boars (detection-mating arena [31]). During expo-
sure to boars, the experimenter checked for estrus by ap-
plying pressure to the back of the gilts to test for a standing
response (back-pressure test [32]). In the presence of a boar,
estrous gilts will stand immobile in response to pressure on
the back [32]. Estrus in gilts was then synchronized by the
administration of 20 mg of oral progesterone (Altrenew;
Regumate, Roussel Uclaf, Paris, France) in feed every day
for at least 18 days [33]. Following the third estrus after syn-
hchronization, catheters were inserted into the anterior
vena cava, via the cephalic vein [34], of 24 gilts. Gilts were
housed in groups of 4 with a space allowance of 1.5 m²
per gilt.

Twelve sexually mature boars (Large White × Land-
race), with known levels of sexual behavior, were housed
in individual stalls that surrounded the detection-mating
arena with a space allowance of 2.3 m² per boar. The level
of sexual behavior of boars had previously been measured
in a series of 15-min tests in which the number of estrous
sows mated and the mean time to mount were recorded.
Six boars of moderate sexual behavior were vasectomized
for use in mating tests to assess the sexual receptivity of
gilts. Vasectomized boars were mated 1–3 times to ensure
that no viable sperm remained in the reproductive tract.
Subsequently, semen was collected and examined under a
microscope to ensure that there were no remaining sperm.
One additional boar was selected for use in a test to assess
the level of proceptivity of the gilts (see below).

The care and experimental use of the animals in this
experiment conformed with the requirements of the Aus-
tralian Prevention of Cruelty to Animals Act 1986 and the
NH&MRC/CSIRO/AAC ‘‘Code of Practice for the Use of
Living Animals in Scientific Investigations.’’

Experimental Design

Gilts were allocated randomly to two treatments (n = 24
per treatment; 12 gilts with catheters per treatment). Gilts
in the negative handling treatment were approached with a
commercial battery-operated stock-moving prodder (3 V,
1.2 A) every 10 sec for 40 sec. If the gilt did not actively
avoid the experimenter, she was given a brief (about 1 sec)
electric shock. Gilts in the control treatment were held in
the treatment pen for 40 sec, but the experimenter did not
interact with the gilt. Gilts were treated individually while
being held in a 3-m × 3-m treatment pen. Treatment was
conducted each day at 1230 h for 7.9 ± 0.5 (mean
± SEM) min to a 3-m × 4-m test pen that was adjacent to a
2-m × 4-m pen containing a mature boar. The amount of time that
the gilt spent within 0.5 m of the pen containing the
boar was recorded [35].

Proceptivity test. Each estrous gilt was introduced for 3
min to a 3-m × 4-m test pen that was adjacent to a 2-m ×
4-m pen containing a mature boar. The time until the gilt
became restless during ejaculation, the duration of ejacula-
tion, and the partner that terminated copulation were recorded [1].

Fear of humans test. The experimenter entered a 3-m ×
3-m octagonal test pen and stood immobile at one side of
the pen throughout a 3-min test. The time until the gilt
approached to within 0.5 m of the experimenter, the total
time that the gilt spent within 0.5 m of the experimenter,
the time until the gilt interacted with the experimenter,
and the number of times the gilt interacted with the experi-
menter were recorded [1].

Measurement of Plasma Concentrations of Cortisol

Samples of blood were collected in syringes containing
EDTA and transferred to blood tubes that were centrifuged
at 3000 rpm for 10 min. Plasma was harvested and stored
at −20°C until analysis. After extraction of the plasma with
dichloromethane, total plasma concentrations of cortisol
were measured in an RIA using hydrocortisone (H-4001;
Sigma Chemical Co., St. Louis, MO) as standard. This RIA
was developed for analysis of fetal sheep plasma [36] and
has since been validated for analysis of pig plasma [27].
Six assays were conducted using 50 μl of plasma with a
mean (± SEM) assay sensitivity of 0.20 ± 0.07 ng/ml and
a range in sensitivities of 0.07–0.52 ng/ml. Samples that
collapsed the sensitivity of an assay were reassayed using
100 μl or 200 μl of sample. The intraassay coefficient of
variation was 10.1% at 39.9 ng/ml, and the interassay co-
efficient of variation was 15.9% at 24.2 ng/ml.

Statistical Analysis

Plasma concentrations of cortisol 15 min and 3–4 h after
exposure to boars in the detection-mating arena. When a gilt
was detected in estrus, her level of sexual receptivity was
assessed in a standard mating test (see below). Gilts were
artificially inseminated 12 h after estrus was first detected
using a standard insemination procedure. Gilts were inse-
minated with mixed, extended, fresh semen (not older than 3
days) from the same two boars, during fencedeline exposure
to boars in the detection-mating arena. A test of proceptiv-
ity was conducted for each gilt 24 h after estrus was first
detected (see below). Once all gilts were out of estrus (5–
18 days after the end of estrus), a test to assess the gilts’
fear of humans was conducted (see below). Gilts were
slaughtered 20–21 days after insemination, and their repro-
ductive tracts were collected. The number of corpora lutea
(CL) and the number and weights of embryos of pregnant
gilts were recorded. If a gilt was not pregnant, the number
of corpora albicantia was recorded.

Procedures

Sexual receptivity (mating) test. Each estrous gilt was
introduced to a vasectomized boar in a 3-m × 3-m octa-
gon test pen. The time from when the boar first attempted
to mount until the gilt displayed a standing response, the
time until the gilt became restless during ejaculation, the
duration of ejaculation, and the partner that terminated copu-
lation were recorded [1].

Proceptivity test. Each estrous gilt was introduced for 3
min to a 3-m × 4-m test pen that was adjacent to a 2-m ×
4-m pen containing a mature boar. The amount of time that
the gilt spent within 0.5 m of the pen containing the
boar was recorded [35].

Fear of humans test. The experimenter entered a 3-m ×
3-m octagonal test pen and stood immobile at one side of
the pen throughout a 3-min test. The time until the gilt
approached to within 0.5 m of the experimenter, the total
time that the gilt spent within 0.5 m of the experimenter,
the time until the gilt interacted with the experimenter,
and the number of times the gilt interacted with the experi-
menter were recorded [1].
the data prior to analysis. The data were transformed for the plasma concentrations of cortisol at both 15 min (log₁₀ transformation) and 3–4 h (square root transformation) after treatment. For all ANOVAs, paired comparisons were made using least significant differences.

For proportional data, comparisons between treatments were made using chi-square analysis. These variables were the number of gilts that became pregnant, the number of gilts that approached and interacted with the experimenter after a significantly (p < 0.05) greater period than did gilts in the negative handling treatment (Fig. 2). Although the plasma concentrations of cortisol varied throughout the 7 days of the experiment, this variation was not related to treatment.

Behavioral Response to Humans

In the behavioral test to assess the level of fear of humans, a significantly (p < 0.05) higher percentage of control than negatively handled gilts interacted with the experimenter (Table 1). Furthermore, control gilts approached and interacted with the experimenter after a significantly (p < 0.01) shorter period than did gilts in the negative handling treatment (Table 1). The mean (± SEM) time that gilts spent within 0.5 m of the experimenter and the number of interactions with the experimenter were significantly (p < 0.05) greater in gilts in the control than in the negative handling treatments (Table 1).
In this experiment, repeated acute stress in gilts in the period before and during estrus did not impair reproduction. On the basis of the proposal of Moberg [11], we had expected that imposing stress in this period would inhibit reproduction. While it is generally accepted that reproduction can be impaired by prolonged activation of the hypothalamic-pituitary adrenal axis (for reviews see [11, 37–39]), there have been few attempts to establish whether or not repeated acute stress will also inhibit reproduction, as we have done in this study. Our results suggest that the repeated activation of the hypothalamic-pituitary adrenal axis in gilts did not critically affect the series of endocrine events that precede, and control, estrus and ovulation.

Negative handling clearly imposed a severe stress on the gilts in this experiment as evidenced both by the extent of activation of the hypothalamic-pituitary adrenal axis and by specific behavioral responses of the gilts. The maximal plasma concentrations of cortisol observed after negative handling were similar to, or higher than, those that have been reported in female pigs in previous studies following a variety of short-term stressors (e.g., mating [40], 1-h confinement in a box and electrical stimulation [21], use of a snout rope [22, 23], relocation and mixing with unfamiliar pigs [24], transportation [25], treadmill exercise [41], and introduction to a boar [26, 27]). Moreover, it is likely that the plasma concentrations of cortisol remained elevated for longer than previously seen following a brief period of stress. The plasma concentrations of cortisol were significantly elevated 3–4 h after negative handling, whereas in previous studies in which brief stressors have been imposed upon pigs, plasma concentrations of cortisol had returned to basal levels within this period (e.g., 1-h confinement in a box and electrical stimulation [21], use of a snout rope [23], relocation [24], treadmill exercise [41], and introduction to a boar [27]). In addition to the adrenal response, it was apparent that gilts that were negatively handled had a higher level of fear of humans than did gilts in the control treatment.

Despite the substantial activation of the hypothalamic-pituitary adrenal axis by repeated negative handling during the period prior to estrus, reproduction was not impaired. This experiment comprehensively investigated the effects of repeated acute stress during the period leading up to ovulation. Thus, it appears that repeated acute stress during this stage of the estrous cycle does not significantly disrupt endocrine events that are critical to estrus and ovulation in gilts. Moreover, the effects of repeated acute stress have not been adequately investigated in females of other species. Although some studies have implied that acute stress can affect reproduction in females, these either imposed the stress for longer than we did and/or did not impose the stress repeatedly, as we did. For example, laparoscopies [12], shearing [13, 14], confinement [15], and wetting-stress [16] in sheep, restraint in monkeys [18], and immobilization in rats [19] have been shown to inhibit certain aspects of reproduction in females, but all these treatments were imposed for substantially longer periods than our negative handling treatment. Stoebel and Moberg [17] stressed cows twice daily for 3.5 days during the follicular phase of the estrous cycle, but the duration of their stress treatment was 15 min each time. Even with this treatment, estrus was normal in all 7 cows, and 5 of the 7 treated cows had a normal LH surge [17]. While it is clear that prolonged activation of the hypothalamic-pituitary adrenal axis can impair reproduction in females [11, 37–39], our results suggest that the repeated acute stimulation of this axis might not impair ovulation and/or estrus in females. Indeed, it appears that cortisol needs to be elevated for periods longer than 3–4 h to alter estrus and ovulation in gilts.

That repeated acute stress did not impair estrus or ovulation in gilts suggests that the factors controlling these aspects of reproduction are resistant to short-term challenges that activate the hypothalamic-pituitary adrenal axis. Activation of the adrenal glands in this manner probably represents a normal response to maintain homeostasis. This finding has important practical implications because it suggests that management practices in the intensive production of pigs that involve short-term intervention with female pigs, and the consequent increase in the secretion of cortisol, are unlikely to have detrimental effects on reproduction.

In summary, repeated acute activation of the hypothalamic-pituitary adrenal axis in the period leading up to estrus and ovulation did not impair reproductive performance in
gilts. The negative handling treatment resulted in substantial elevations in plasma concentrations of cortisol for periods of at least 3–4 h and induced a higher level of fear of humans than in control gilts. Nonetheless, none of the parameters of reproduction or sexual behavior were affected by negative handling. It appears that the factors that control estrus and ovulation in gilts are resistant to activation of the hypothalamo-pituitary adrenal axis for short periods and that more long-term activation is required to inhibit reproduction.

ACKNOWLEDGMENTS

We thank the late Dr. Ron Parr for his help and advice. We also thank Dr. Ben Canny for helpful discussion and Bruce Schirmer, Samantha Borg, Lisa Newman, and Tina Chamberlain for their assistance.

REFERENCES