Stress and Reproduction

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What is stress as it relates to reproduction?

- Imbalance of homeostasis in the struggle of the individual between maintaining itself within its environment and perpetuating its genetics
Classic indicators of stress

- Elevated blood plasma levels
  - ACTH
  - Cortisol
  - Adrenalines

Clinically...
- Neutrophilia
- Lymphopenia
- Hyperglycemia
How and to whom does it manifest?

- Many molecular mechanisms and pathways (HPA/HPG, immune, oxidative)
- Both sexes at all reproductive stages of life
Qualifiers of stress response

► Previous experience (adaptation to stimuli)

► Genetics (Bos indicus vs. Bos taurus)

► Species (human complexity)

► Age (last ditch effort)

- Lower levels of glucocorticoids released in response to handling stress decreases with age in nesting terns (Heidlinger 2006)
Qualifiers of stress response

► Physiologic status (i.e., negative energy balance)

► Gender
  - sex hormone “neuroactive steroid” influences on pain response
    - Higher glucocorticoid levels in females than males after HPA axis stimulation (Aloisi 2006)*

► Timing, type, and duration of stress stimulus
  - Different stimuli result in different physiologic responses i.e., Blood cortisol, fatty acids, and glucose blood levels (Odio 1985)
    - Example: cortisol levels are higher for immobilization than footshock in rats
**WHAT** is stress?

**Broad categories of stress**

1) **Environmental** i.e. thermal & oxidative?

2) **Physiologic** i.e. nutritional deficiency

3) **Psychological/psychosocial** i.e. overcrowding
**HOW**: Stress pathways (HPA)

- **Hypothalamus (PVN)**
  - Stress event
  - Release of neurotransmitters (i.e., NE, cholecystokinin, serotonin)

- **CRH, AVP**

- **Ant. pituitary**
  - ACTH, β endorphins

- **Cortisol**
  - Release of neurotransmitters (i.e., NE, cholecystokinin, serotonin)

- **Adrenal gland**
  - Catecholamines
Stress pathways (HPG/HPA integration)

►► Hypothalamus

(PVN) GnRH

CRH

adrenal

catecholamines

cortisol

Ant. pituitary

GnRH

ACTH

- ?

LH  FSH

E2

testosterone

Gonads

Repro tracts abundant in receptors for CRH and cortisol
Complicated by...

- Glucocorticoids negative feedback at each level of HPA axis
  - Multiple receptor types: have both genomic and non-genomic effects

- ACTH can suppress pit. responsiveness to GnRH in ovary intact ewes

- β endorphins can directly affect GnRH neuronal activity in some studies but actual role undetermined

- Catecholamines also involved but to what extent

- HPG affects HPA? Estrogens bind to receptors on the CRH gene, increasing CRH activity (Aloisis 2005)

- CRH
  - direct roles in inhibiting GnRH release not well defined
  - decreases testosterone synthesis in rat leydig cells
  - receptors present in many tissues, incl. placenta and gonads
  - actions at local levels unknown? (Breen 2006)
Relevant findings

► Suppression of LH pulse freq with cortisol treatment in women

► Cortisol inhibits pulsatile LH secretion in ovariectomized ewes (within 0.5-2h)
  - Without reducing GnRH secretion

► Women with chronic anovulation and lower implantation rates have higher serum cortisol and CRH concentrations than healthy controls

  (Breen 2006, Gallinelli 2001)
WHEN:
Stress effects at multiple stages

► Pre-fertilization
  - Copulatory behavior
  - Gamete maturation (females more crucial?)

► Gestational
  - Pre-implantation
  - Placentation and beyond
  - Organogenesis

► Post-partum behavior
  - Offspring
  - Maternal
Stress at pre-fertilization

- Copulatory behavior

- depression, anxiety and chronic stress may interfere with central and peripheral pathways of the sexual response (decreased sex drive)
  - zoo animals decreased reproductive capacity associated with captivity
  - Classically, decreased estrus in heat stressed cattle
Stress at pre-fertilization

- **Effect on testosterone**
  - Acute treatment of ACTH inhibits testosterone by bovine, ovine and primate testis
  - In contrast, single injection ACTH stimulates testosterone from boar, rabbit testis
  - Glucocorticoid receptor present in Leydig cells
    - Cortisol can lower LH receptor number
    - Resulting decreased androgen synthesis
Stress at pre-fertilization

► Heat stress dampens estrus behavior in cattle

► Effect
  ▪ Glucocorticoid treatment decreases gonadotropin secretion and induction of aromatase activity
  ▪ Estrogen decrease
  ▪ Protective mechanisms (cortisol-metabolizing enzymes in repro tissues)
Stress at pre-fertilization

Gamete maturation via HPA/HPG interference

- Males: disturbance of spermatogenesis, decreased sperm fertility parameters

- Females: disturbance of folliculogenesis
  - Inhibits LH pulses, subsequent E2 and thus, LH/FSH surge
  - Also may inhibit gonadotropin responsiveness in granulosa cells, given glucocorticoid receptor presence (Breen 2006)
Stress effects in gestation

- **Pre-implantation stages**
  - Early embryonic loss in livestock and mice

- **Placentation and beyond**
  - Placenta: abundant receptors for corticosteroids and catecholamines
  - Humans:
    - increased incidence of spontaneous abortion, preterm delivery and low birth weight
    - increased uterine a. resistance with high anxiety scores (Mulder 2002)
  - Cattle, sheep in heat stress last 2/3 gest.
    - exhibit reduced utine and umbilical blood flows, resulting reduced fetal oxygen, nutrients and fetal size (VEGF and RmRNAs) (Reynolds 2005)

- **Organogenesis**
  - adrenal
Stress effects post-partum

- Maternal behavior

  - Stress during gestation alters postpartum maternal care and the development of the offspring in a rodent model

    - Possible mechanism: cortisol alters oxytocin receptor expression (Champagne 2006)
Stress effects post-partum

- Behavior of offspring
  - prenatal stress induces developmental and behavioral disorders
    - Female progeny bad mothers too (Mulder 2002)
  - prenatal stress had a long-term negative effect on sexual behavior in rats (Gerardin 2005)
Stress ≠ infertility

- Cortisol required for normal parturition
  - Induction
  - PTSD: low profile cortisol, more complications (Seng 2005)

- Certain oxidative stress necessary for disulfide bond formation and ovulation

- Acute stress causes increase in LH/testosterone in monkey and boar
1) Environmental stress

- **Heat**
- **Oxidative**
  - In close connection with heat stress and metabolic stress
- **Toxicants**
Environmental stress: heat

► MALES
  ▪ Spermatogenesis

► FEMALES
  ▪ Reproductive loss
    ► Widely known in cattle
      ▪ Reduced intensity and duration of estrus
      ▪ Reduced oocyte quality (Rocha 1998)
      ▪ Reduced pregnancy rates from AI or natural service during periods of hot weather

► heat stress reduces the developmental ability of embryos (mice: Ozawa 2002; sheep and cattle: Rutledge 2001)

► cattle, sheep models subjected to chronic heat stress result in decreased uterine, umbilical blood flow (Reynolds 2005)

Environmental stress

► What about cold?
  ▪ Extremes in climate alter energy transfer with deleterious effects on reproduction
    ► possibly through cortisol mediation and increased caloric need
  ► cold as stress inducer in mice studies
  ► Most obvious in calf scrotal frostbite, may result in infertility
COLD and maternal stress

- repeated cold stress (rapid freq. temp. changes) in rats prenatally produced offspring with higher startle responses (Tazumi 2005)
Environmental stress: oxidative

- Involves production of ROS (O$_2^-$, H$_2$O$_2$, OH$^*$)

- Not all bad
  - Required for many processes (sperm DNA condensation, ovulation)

- Increased ROS implicated in heat stress *in vivo and in vitro*

- Lipid, protein, DNA damage
  - Leads to cellular injury
  - Impedes, inhibits embryonic development (Rutledge 2001)

Presence of ROS (i.e. from heat)

NORMAL

Lack of adequate ROS reduction, scavaging system

DEGENERATING

Ozawa 2002, FAO,
Maternally heat stressed embryos

![Graph showing GSH levels in zygote and two-cell stages under control (C) and heat stress (HS) conditions.](Image)

![Images of embryos showing high and low levels of a fluorescent substance.](Image)

Ozawa 2002
Environmental stress: oxidative

- More detrimental \textit{in vitro} than \textit{in vivo}*

- Major concern in ART
  - superoxide dismutase (SOD), catalase, vitamins (A, C and E), glutathione and pyruvate
  - Inhibition of glutathione reduces thermotolerance [increase ROS] of embryos (interference with genome activation?)
  - Female SOD knockouts infertile (Fujii 2005)

Ozawa 2002
2) Physiological stress

- Metabolic
- Inflammatory
Physiological Stress

- Nutritional and metabolic stress
  - Impact the reproductive system
  - Undernutrition results in a compromised reproductive function
  - Metabolic signals play a critical role in response to stress
Nutritional Stress

Nutritional stress factors:
- Altered body fat ratio
- Weight Loss
- Nutrient deficiency
- Eating disorders

Models:
- Postpartum Dairy Cows
- Rats, Pigs, and Sheep
- Undernourished Women
Nutritional and Metabolic Signals

- Glucose is main energy source
- If glucose is not available, body shifts to maintain homeostasis
- Body attempts to produce glucose through alternate forms of metabolism
- Mechanisms impaired during times of stress
Metabolic Stress

► Neuro-hormonal axis works to maintain energy balance

► Serum glucose and insulin provide feedback to the brain regarding fuel availability

► Adiponectin and leptin are hormones produced by adipocytes that help regulate fuel storage

► Thyroid axis is responsible for adjusting basal metabolic rate

(Basanta-Henry 2006)
Metabolic Factors on Ovarian Function

Webb et al. 2004
Metabolic - Stress

- The GnRH pulse is very sensitive to stress and metabolic factors.

- GnRH pulse is highly sensitive to insults:
  - Weight loss
  - Decreased energy availability
  - Altered body fat ratio

- Stress disrupts pulsatile GnRH secretion

- GnRH secretion affects pituitary secretion:
  - LH and FSH
  - Ovarian stimulation
  - Estrogen production

(Lucy 2000, Basanta-Henry 2006)
Regaining Homeostasis:

1st) Energy in milk production

2nd) Body Condition

3rd) Reproductive Process

Postpartum Dairy Cows

- **Shifting metabolic status**
  - ↓ IGF-1 - Liver
  - ↓ Insulin - Pancreas
  - ↓ Leptin - Adipose tissue

*These signals act on hypothalamus to convey information to ovary to release/inhibit FSH and LH*

(Lucy 2000)
Postpartum Dairy Cows

- Lactating cows
  - IGF-1 – gradually increases
    (can remain low)
  - Insulin – gradually increase
  - Leptin – remains low

(Lucy 2000)
Postpartum Dairy Cows

- Time to 1st ovulation is increased
  - Dependent upon LH re-establishment

- Smaller CLs

- Decrease of Progesterone

- Less estrogenic dominant follicles

Postpartum

- Increase in LH
  - appropriate body mass

- Increase LH stimulates dominant follicle maturation

What does this mean?

► Selecting for increased milk production
  - Increasing stress on system
  - Decreasing nutrients
  - Decreasing reproduction

► Nutritional needs shift from regaining body condition to milk production

Postpartum Dairy Cows

- Time to 1st ovulation is increased
  - Dependent upon LH re-establishment

- Smaller CLs

- Decrease of Progesterone

- Less estrogenic dominant follicles

Postpartum Dairy Cows

- High protein diet or adding lipids to diet?

- Adding Lipids
  - Lipid supplementation during 1st follicular wave
  - Cows unaffected

- High protein diet supports milk production
  - Does not reactivate ovulation
  - Can increase uterine pH
  - Increase blood concentrations of ammonia and urea
  - Increased plasma urea nitrogen = decreased fertility

* Increased milk production leads to cows with less adipose tissue and greater infertility

(Butler 2000)
Nutritional Imbalance - Rat Model

- Restricted protein 2wks prior to mating throughout gestation
  - Modest restriction (9% vs. 18% protein)

- Upregulation of glycolysis
  - stress response, 9.5-10.5 days post fertilization

- Increased offspring insulin resistance

- Increased offspring blood pressure

(Lesse 2002)
Feed Restriction on Cyclic Gilts

- Moderate Feed Restriction
  - 1\text{st} or 2\text{nd} week of estrus

- Lower embryonic survival

- Unaltered IGF-1, T3, or Insulin

- Lowered Progesterone

- Improvement with insulin injections ??

(Almeida 2000, 2001)
Cyclic Gilts

- **Insulin to counteract feed restriction**
  - Feed restricted 2\textsuperscript{nd} wk/plus insulin
  - Insulin (0.4IU/kg body weight 2x’s/day)
  - Influences steroid oogenic activity?
  - Affected follicular maturation
  - Greater development of oocytes
  - Decreased detrimental affects (hyperinsulinemia)

- **Progesterone shots**
  - Early pregnancy
  - Counteracted nutritional induced embryonic loss

(Almedia 2000, 2001)
Lactating Sows

- Feed restriction late-lactation
  - Decrease embryonic survival
  - Development of sex ratio skewing
  - Treatment sows had ½ the control sow’s feed (n=17/group)

- Decreased litter growth

- Decreased sow fertility

- Decreased embryonic survival

- Trend towards male based litters
  - not desirable in pigs (61% males vs. 54% in controls)

- Improper methylation of genes on X chromo
  - feed restriction decreases female embryos

- Uterine and embryonic environment skew in favor of males

(Vinsky 2006)
Sheep Model

- Decreased late gestational nutrition
  - 120hrs. Complete fasting
  - Fasting between days 130-135 of gestation

- Insulin-signaling pathway most responsive to stress?

- Glucose decreased 50%

- Intrauterine growth restriction

- Postnatal insulin resistance – nutritional stress

- Decreases sensitivity to steroid hormones

(Shen 2005)
Sheep Model

- Undernutrition
  - ½ feed at day 28 pregnancy
- Oxidative base damages to DNA of fetal oogonia
- Decreased number of follicles
- Tumor suppressor protein p53
  - Arrests oogonia
  - Allowing for repair and proof-reading of DNA
- p53 found on ovaries of restricted ewes

(Murdoch 2003)
Women

►► Energy investment
  ▪ Poor condition – investment unwise

►► Extreme stress
  ▪ Early parturition
  ▪ Growth restriction
  ▪ Increased morbidity and mortality

(Wiley-Less 2005)
Functional Hypothalamic Amenorrhea (FHA)

- Stress Induced Amenorrhea
- Exercise Induced Amenorrhea
- Eating disorders cause a range of menstrual disorders/infertility
- Amenorrhea due to chronic low energy intake
- Metabolic factors mediate reproductive adaptations

FHA Women

- No significant change in serum glucose levels
  - Elevated cortisol caused other energy sources to be converted into glucose

- Low Insulin levels
  - Chronic undernutrition results in depletion of pancreatic glucose stores
  - Elevated cortisol levels

FHA Women

- Reduced secretion of:
  - GnRH
  - LH
  - FSH

- Increased secretion of:
  - Cortisol
  - IGFBP-1 (limits activity of IGF-1)
  - GH

FHA Women

► Energy imbalance major mechanism

► Inadequate leptin production
  ▪ Stimulate the release of ACTH & endogenous opioids

► Increased cortisol

► Low estrogen

► HPA axis displays altered feedback inhibition
  ▪ increased cortisol does not alter CRH level

► HPT axis displays low levels of T3 and T4
  ▪ w/o compensatory rises in TSH

FHA Women

- Chronic hypoinsulinemia
  - decreased insulin sensitivity
  - may affect the leptin and adiponectin production

- Leptin and adiponectin provide feedback to hypothalamus
  - Decreases GnRH

Gambia Rural Women Study

- 40yr study
- Poor nutrition
- Low birth weight
- Low blood pressure
- Low cardiovascular disease

- Women in unnourished environments
  - Smaller individuals
  - Produced smaller babies

  **Adaptation to stressful environment**

(Wiley-Less 2005)
Physiological stress: inflammatory

►► Immune mediators of stress

- Interleukin receptors at all levels of HPA and HPG axes, affecting hormonal release (gonadotropins directly) (Breen 2006)

- Early pregnancy loss attributed to a stress-induced TNF-α pathway

- Bacterial endotoxins block or delay LH surge (rat, cow, sheep, monkey)

- And reduce luteal phase progesterone secretion (sheep, monkey)
Psychological/psychosocial stress

►► Human studies

- conflicting results
  - Some find no effects of psycho stress on IVF success rate (markers inadequate?)
  - Recent studies suggest links (cause or effect?)
    - High anticipatory cortisol levels prior to OR and ET result in lower pregnancy rates
- Behavioral treatment shown effective (to what degree?)

- mental stress negatively affected semen quality (Eskiocak 2005)
  - decreased levels of glutathione, insufficient SOD (ROS overcomes)
  - inhibition of the conversion of androstenedione into testosterone in Leydig cells on account of higher adrenocorticotropic hormone (ACTH) and cortisol levels

Campagne 2006
### Table I. Markers for stress

<table>
<thead>
<tr>
<th>Substance/method</th>
<th>Relevance for acute/chronic stress</th>
<th>Relevance as stress marker for IVF outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenaline</td>
<td>High</td>
<td>High (at OR, ET)</td>
</tr>
<tr>
<td>Noradrenaline</td>
<td>High</td>
<td>High (only at ET)</td>
</tr>
<tr>
<td>ACTH</td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td>Amylase</td>
<td>Variable</td>
<td>Questioned</td>
</tr>
<tr>
<td>Dehydroepiandrosterone</td>
<td>High</td>
<td>?</td>
</tr>
<tr>
<td>Cortisol</td>
<td>Variable</td>
<td>Site-dependent</td>
</tr>
<tr>
<td>Estrogen</td>
<td>Variable</td>
<td>?</td>
</tr>
<tr>
<td>Prolactin</td>
<td>Probable</td>
<td>Probable</td>
</tr>
<tr>
<td>Progesterone/allopregnanolone</td>
<td>Variable</td>
<td>Questioned</td>
</tr>
<tr>
<td>LH</td>
<td>Probable</td>
<td>Probable</td>
</tr>
<tr>
<td>Vasopressin</td>
<td>High</td>
<td>?</td>
</tr>
<tr>
<td>NK cells</td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td>Cardiovascular reaction to provoked stress</td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td>Depression (even subclinical)</td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td>High active coping</td>
<td>High</td>
<td>Probable</td>
</tr>
<tr>
<td>High avoidance</td>
<td>High</td>
<td>Probable</td>
</tr>
<tr>
<td>High expression of emotion</td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td>State anxiety</td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td>State-anxiety self-report</td>
<td>Questioned</td>
<td>Questioned</td>
</tr>
<tr>
<td>Trait anxiety</td>
<td>Some</td>
<td>Some</td>
</tr>
<tr>
<td>Trait-anxiety self-report</td>
<td>Questioned</td>
<td>Questioned</td>
</tr>
</tbody>
</table>

ACTH, adrenocorticotropic hormone; ET, embryo transfer; NK, natural killer; OR, oocyte retrieval. See text for references. Important differences between female and male factors have been found.
Investigating further...

- Neuroendocrine sites, cell types and better characterize mechanisms of cortisol effect on decreased pituitary responsiveness
- Chronic vs. acute effects (diet/schedules vs. counseling)
- Methods of stress management and control
- Appropriate models
  - overcrowding
  - isolation
  - transportation
  - restraint/captivity
  - Forced swimming
  - shock
  - cold/heat stress
Stress management

► Managerially (livestock)
  - Diet, housing, temperature control

► Pharmacologically
  - Work in progress (i.e. anti-oxidants in oxidative stress)

► Psychiatrically (human ART)
  - Stress levels/test responses as predictors of IVF outcomes
Addressing Stress

- Circumventing heat stress in cattle
  - Fixed-time insemination synchronization and embryo transfer
  - Fan-misting at hottest periods of day
  - Timed breeding season and gestation around heat
  - Genetic influence