

Study of the pituitary–gonadal axis in the proestrus phase in adult female rats subjected to social isolation

Nehal M. Bahgat

Department of Physiology, Faculty of Medicine,
Ain Shams University, Cairo, Egypt

Correspondence to Nehal M. Bahgat, Department
of Physiology, Faculty of Medicine, Ain Shams
University, 11733 Cairo, Egypt
Tel: +20 100 565 2386; fax: +20 2683 7673;
e-mail: nehalgamil@yahoo.com

Received 8 August 2012

Accepted 4 September 2012

Journal of the Arab Society for Medical Research
2012, 7:86–91

Background/aim

Social isolation is a type of stress that might adversely affect sex cycles in both animals and humans. The present study was planned to investigate the pituitary–gonadal axis in the proestrus phase of estrous cycle in adult female rats subjected to social isolation stress for 8 weeks.

Materials and methods

Twenty Sprague–Dawley adult female rats were divided into two experimental groups: a control group ($n=10$) and a socially isolated group (SI, $n=10$). Throughout the study, all rats were monitored for body weight and food intake. After 8 weeks, rats were sacrificed in the proestrus phase of estrous cycle. All rats were examined for final body weight, rectal temperature, hematocrit value, and serum levels of follicle stimulating hormone, luteinizing hormones, prolactin hormone, 17- β estradiol, and progesterone in addition to histological examination of the ovaries.

Results

The results of the present study showed that the SI group had significant decrease in their final body weights and their serum levels of 17- β estradiol and progesterone, whereas the serum level of prolactin was significantly increased. Histological examination of SI rat ovaries showed fewer growing ovarian follicles and numerous atretic ones compared to control rat ovaries.

Conclusion

These findings indicate that social isolation might result in depressed ovarian function in adult female rats.

Keywords:

estrogen, gonadotropins, proestrus phase, progesterone, prolactin, social isolation stress

J Arab Soc Med Res 7:86–91
© 2012 The Arab Society for Medical Research
1687-4293

Introduction

Social isolation is the lack of contact and interaction with other individuals [1]. Subjectively, it is the feeling of loneliness or lack of companionship. Loneliness is the perception of being alone and can be experienced even when one is in contact with others [1].

Social isolation is a type of stress [2] that might permeate the life of any individual. The causes of social isolation stress might be loss of a spouse, low self-esteem, physical disability, serious health problems, stigmatizing chronic illnesses such as AIDS, and implementation of the penalty of solitary confinement. Social isolation has been reported to be associated with anxiety, mood depression, increased incidence of smoking as well as greater risk of cocaine addiction [3].

Many observations have indicated that social isolation might adversely affect ovulation. Subordinate female common marmosets were observed to have hypoestrogenic anovulatory cycles [4]. However, when their housing conditions were changed, they were found to undergo their first ovulation [5]. Adult female rats subjected to social isolation from young adulthood to late

middle age showed early ovarian senescence with only secondary and atretic follicles at necropsy [6]. Hermes and McClintock [7] have reported that isolated female rats had fewer tertiary follicles than group-housed animals and that corpora lutea, indicating successful ovulation, were entirely absent in the isolates. In humans, the findings were not different from animals observations were in agreement with those in animals. Allsworth *et al.* [8] have reported that incarcerated women had a high prevalence of amenorrhea and menstrual irregularities.

Ovulation occurs in the young adult laboratory rats every 4–5 days throughout the year [9]. The durations of the individual components of the estrous cycle are 12–14 h for proestrus; 25–27 h for estrus; 6–8 h for metestrus; and 55–57 h for diestrus [10–13]. Ovulation was reported to occur in the estrus phase between 08:00 and 10:00 h [14].

The cyclic changes that occur in the female reproductive system and ovulation are regulated by hypothalamic–pituitary–ovarian hormones. In the proestrus phase, plasma levels of follicle stimulating hormone (FSH), luteinizing hormone (LH), prolactin (PRL), estrogen, and progesterone (PRG) have been reported to increase [15].

Increased plasma estrogen level by the growing follicles induces the preovulatory LH surge that leads to ovulation [16].

The timing of the 17- β estradiol (E2) peak during the proestrus phase has been debatable in the previous literature. E2 was reported to peak in ovarian venous blood between 10:00 and 12:00 h [17], whereas it was reported by Nequin *et al.* [14] to peak in serum at 06:00 h. However, Smith *et al.* [18] reported a persistently high E2 level between 08:00 and 16:00 h on the proestrus day. The increase in LH and FSH follows the estrogen peak and occurs between 16:00 and 18:00 h according to Gay *et al.* [19]. Normally, the PRL level increases in the proestrus phase, reaching its peak at 20:00 h [14]. PRG secretion increases at 17:00 h, followed by a significant decrease in estrogen secretion between 21:00 and 23:00 h [17]. This increase in the PRG level is directly involved in induction of female reproductive behavior [20], ovulation [21], and formation of corpus luteum [22].

An experimental study of social isolation stress has the advantage of lack of interference of stress-associated behaviors such as smoking or drug addiction, which might affect the results. Therefore, the present study was carried out to investigate the changes in the pituitary/gonadal axis in the proestrus phase of the sex cycle in socially isolated adult female rats.

Materials and methods

Experimental animals

The present study was carried out on 20 adult female Sprague–Dawley rats weighing 160–200 g at the start of the experiment. Rats were purchased from Ophthalmic Diseases Research Institute, Giza, Egypt. Rats were maintained in the Physiology Department Animal House, Faculty of Medicine, Ain Shams University, under standard conditions of boarding, at room temperature, $22 \pm 1^\circ\text{C}$. Regular meals were introduced daily at 16:00 h. Rats were fed *ad libitum* water and the standard rat chow diet (AIN-93M diet formulated for adult rodents) prepared according to the National Research Council (NRC) [23] and Reeves *et al.* [24]. The study was approved by the Ain Shams Faculty of Medicine Research Ethics Committee.

Experimental procedure

Rats were housed (3–4 rats/cage) in plastic cages (50 $\times$ 28 $\times$ 16 cm) with standard stainless-steel lids and wood chip bedding for 2 weeks for acclimatization. Vaginal smears were taken daily and rats showing three consecutive regular 4-day cycles were included in the study. Social isolation was carried out by housing 10 rats individually in plastic cages (32 $\times$ 18 $\times$ 15 cm) according to Apter and Eriksson [25]. Experimental rat groups were as follows:

- (1) Control rat group (C; $n = 10$) housed in groups (3–4 rats/cage).
- (2) Socially isolated rat group (SI; $n = 10$) housed individually.

Throughout the study, all rats were subjected to estimation of daily food intake as well as body weight and rectal temperature weekly.

After 8 weeks, vaginal swabs were obtained from overnight fasted rats between 10:00 and 12:00 h. to determine the phase of the estrous cycle. To obtain a vaginal swab, cotton wool swabs were soaked in normal saline and then introduced gently into the vagina according to Marcondes *et al.* [26] with modification. The obtained swab was then spread on a clean glass slide. The characterization of cell types in the swab was determined using the $\times 40$ objective lens. The determination of the estrous cycle phase was carried out on the basis of the proportion between different cell types in the swab using the $\times 10$ objective lens. The cell types are nucleated epithelial cells, anucleated cornified cells, and leukocytes; the proestrus phase is characterized by the predominance of nucleated epithelial cells [26].

Rats in the proestrus phase were weighed and then rectal temperature was measured using a medical thermometer. Rats were anesthetized with sodium thiopental (40 mg/kg intraperitoneally). A midline abdominal incision was made, then the abdominal aorta was exposed, and blood samples were collected as follows:

- (1) Blood (1 ml) was collected in plastic tubes coated with K2 EDTA for estimation of the hematocrit value (Ht) by SFRI blood cell counter H18 (SFRI Medical Diagnostics, Saint Jean D'Ilac, France).
- (2) The remaining blood was collected in clean plastic tubes and centrifuged at 3000 rpm for 15 min for the separation of sera, which were stored at -80°C till used for biochemical analysis. Serum levels of FSH, LH, PRL, and E2 were determined by ELISA kits for rats from EIAab Co. (Wuhan, China). Serum PRG was determined using PRG ELISA kits for rats from MyBioSource Co. (San Diego, USA).

The ovaries were excised and then kept in 10% formalin for histological examinations, dehydrated, cleared in zylol, and embedded in parablax. Paraffin sections were cut serially at 5 μm thickness and stained by H&E as described by Drury and Wallington [27].

Statistical analysis

All statistical data and significance tests were carried out using the statistical package for social science (SPSS Inc., IBM, New York, USA) version 15.0 according to Armitage and Berry [28]. Statistical significance was determined using Student's *t*-test for unpaired data. Correlations and lines of regression were calculated by linear regression analysis using the least square method. A probability of *P* less than 0.05 was considered statistically significant. All data were expressed as mean $\pm$ SEM.

Results

Initial body weights were comparable between the SI and the C rat groups, whereas the final body weights were

significantly lower in the SI group ($P < 0.01$) compared with the C group. However, the average daily food intake, rectal temperature, and Ht value were not significantly different between the two experimental rat groups (Table 1).

Biochemical analysis of the different studied hormones showed that gonadotrophic hormones (FSH and LH) were not significantly different between SI and C rat groups. However, PRL hormone increased significantly ($P < 0.01$) in the SI group compared with the C group, whereas estrogen and PRG hormones decreased significantly ($P < 0.01$) in the SI group compared to the C group (Figs 1 and 2).

Correlation study showed a significant negative correlation between PRL and both gonadal hormones (E2 and PRG) in the C rat group. However, in the SI rat group, the correlation became insignificant (Table 2).

Histological examination of the ovary showed the presence of multiple growing ovarian follicles and corpus luteum in the C rat group (Fig. 3), whereas in the SI rat group, there was only one growing ovarian follicle, one corpus luteum, and numerous atretic follicles (Fig. 4).

Discussion

Social isolation is considered a type of stress for animal species that have intimate social interactions such as rats. In the present study, SI rats showed weight loss, although the average daily food intake was not significantly changed from the control rats. Weight loss might be explained by increased metabolic rate because of stress-induced activation of the hypothalamo-pituitary adrenal axis (HPA) [29]. Increased glucocorticoid production by adrenal glands would enhance the calorogenic effect of catecholamines through its permissive action. Another possible reason might be impaired food digestion and absorption in SI rats because of stress-induced gastrointestinal dysfunction [30,31].

Hormonal assay showed unchanged levels of gonadotrophic hormones between the two experimental rat groups. However, PRL hormone increased, whereas estrogen and PRG hormones decreased significantly in SI rats compared to the controls. Shaikh [17] reported decrease in the Ht value between 10:00–12:00 h and 21:00–23:00 h in the proestrus phase because of fluid retention by steroids. In the present study, the insignificant change in the Ht value excludes the possibility that these hormonal changes were because of hemodilution or hemoconcentration.

PRL hormone has been reported by many authors to be a hormone of stress [32–35]. It has been reported that PRL and corticosterone levels showed a high positive correlation during stress [36]. This finding indicates that the neuronal circuits involved in regulation of the physiological response to stress stimulate the HPA axis as well as PRL secretion. The mechanisms underlying PRL release in stress seem to be regulated at multiple levels. Jahn and

Deis [37] excluded dopaminergic and serotonergic pathways from mediating stress-induced PRL release, whereas it was attributed by Meyerhoff *et al.* [38] to the stress-induced increase in β -endorphin, which has a facilitatory effect on PRL secretion [39]. Corticotropin releasing hormone was suggested by Akema [40] to induce PRL release in acute stress possibly by an undefined stress mediator. Figueiredo *et al.* [29] have reported increased expression of mRNA of immediate early gene *c-fos* – a marker of neuronal activation – in the cingulate cortex, hippocampus, and medial amygdale in response to stress. All these areas have been reported to play important roles in the HPA response to stress [29] and to influence PRL secretion during the estrous cycle, pregnancy, and pseudopregnancy [41]. The physiological role of PRL in stress is not fully understood, but it might be related to its immune modulatory [32], osmoregulatory [42], angiogenic [43], or neurogenic [44] functions.

In rodents, PRL remains low throughout the estrous cycle, except in the evening of proestrus phase in which PRL level shows preovulatory peak after the LH surge [45]. The reason for this PRL surge was reported to be because of the proestrus increase in the estradiol level [46]. PRG has also been reported to advance the PRL surge in proestrus and to increase lactotrophs' sensitivity to estradiol [47,48]. The significance of the proestrus PRL surge is not clear, but in rodents, PRL exerts either a luteotrophic action after mating or a luteolytic action in the absence of a mating stimulus [49]. Whether the PRL level in SI rats in the present study was persistently elevated throughout the estrous cycle or showed premature elevation in the morning of proestrus is difficult to predict in the context of the present study and requires a thorough follow-up throughout the cycle to determine the changes in its secretion.

In the present study, gonadotrophic hormone levels were comparable between SI and C rats, which is not in agreement with the findings of Cameron [50] and Saltzman *et al.* [4], possibly because their researches were carried out on primates. The unchanged levels of gonadotrophic hormones between SI and C rats might explain the presence of a growing ovarian follicle in the SI rat ovary. However, the observable decrease in the number of growing follicles in the ovaries of SI rat group indicates that the response of the SI rat ovaries to gonadotrophic hormones was reduced. A possible cause of depressed ovarian function in the SI rats might be the high PRL level that was found to interfere with FSH-induced aromatase activity in cultured rat granulosa cells [51,52]. However, in the study carried out by Shimizu *et al.* [53], heat stress was also found to inhibit the expression of gonadotropin receptors in granulosa cells and to attenuate the estrogenic activity of growing follicles in immature rats injected with PMSG and that granulosa cells of heat-stressed follicles were more susceptible to apoptosis than control follicles. Whether social isolation for a long duration would exert similar effects in adult female rat ovarian follicles is a matter of speculation that requires further investigations to be clarified.

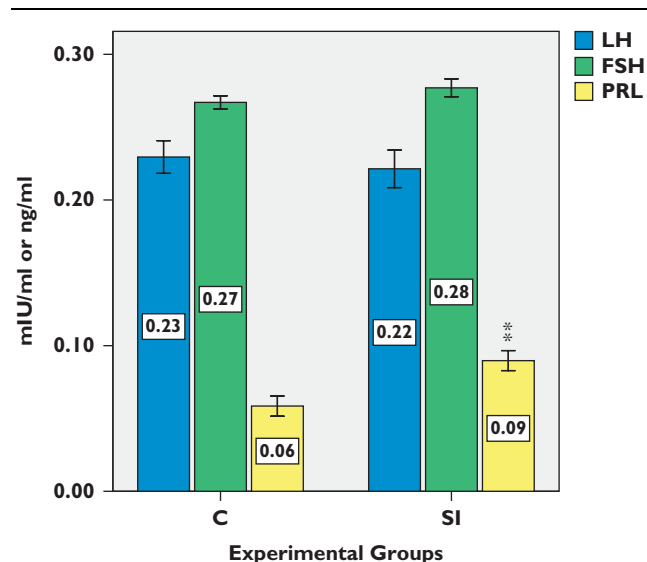
Table 1 Changes in the mean values of the initial and final body weights (g), average daily food intake (g/day), rectal temperature (°C), and hematocrit value (%) in control and socially isolated rat groups

Rat group	IBW (g)	FBW(g)	FI (g/day)	Temperature (°C)	Ht (%)
C (10)	170.50 ± 2.73	206.00 ± 10.84	11.47 ± 0.65	38.06 ± 0.06	45.06 ± 0.72
SI (10)	174.00 ± 4.2	164.00 ± 1.63*	10.48 ± 0.11	38.13 ± 0.13	44.78 ± 2.20

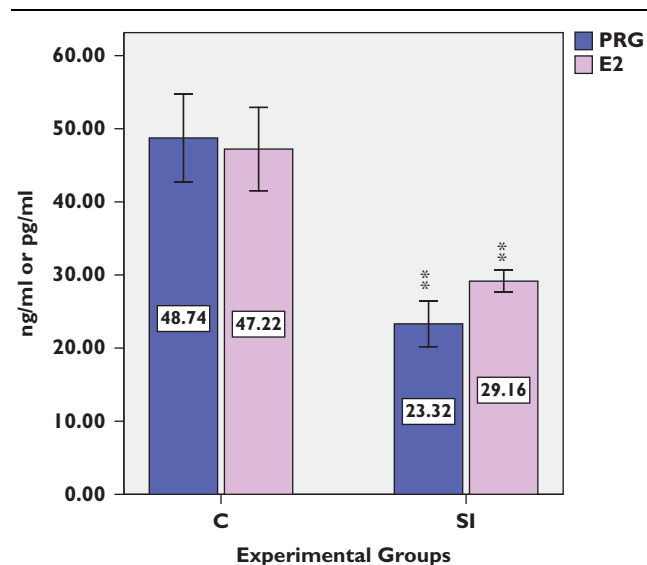
All data are expressed as mean ± SE.

C, control rat group; FBW, final body weight; FI, food intake; Ht, hematocrit; IBW, initial body weight; SI, socially isolated rat group.

* $P < 0.01$ compared with the control group.

Figure 1

Serum levels of follicle stimulating hormone (FSH, mIU/ml), luteinizing hormone (LH, mIU/ml), and prolactin (PRL, ng/ml) in control (C) and socially isolated (SI) rat groups.

Figure 2

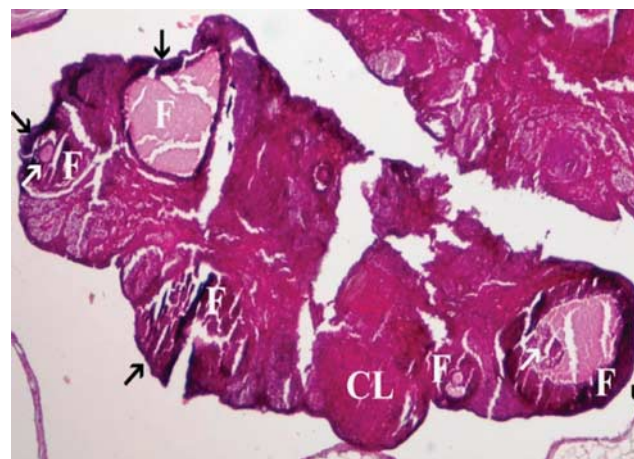
Serum levels of progesterone (PRG, ng/ml) and 17-β estradiol (E2, pg/ml) in control (C) and socially isolated (SI) rat groups.

PRL and the gonadal hormones (E2 and PRG) showed a significant negative correlation in control rats. This observation reflects the presence of a tight regulatory

Table 2 Correlations of serum prolactin (ng/ml) versus 17-β estradiol (pg/ml) and progesterone (ng/ml) in control and socially isolated rat groups

Rat group	PRL vs. E2	PRL vs. PRG
C (n=10)		
<i>r</i>	-0.70	-0.90
<i>P</i>	<0.05	$P < 0.01$
SI (n=10)		
<i>r</i>	0.05	0.16
<i>P</i>	NS	NS

C, control rat group; E2, 17-β estradiol; *n*, is the number of rats; NS, not significant; PRG, progesterone; PRL, prolactin; SI, socially isolated rat group.

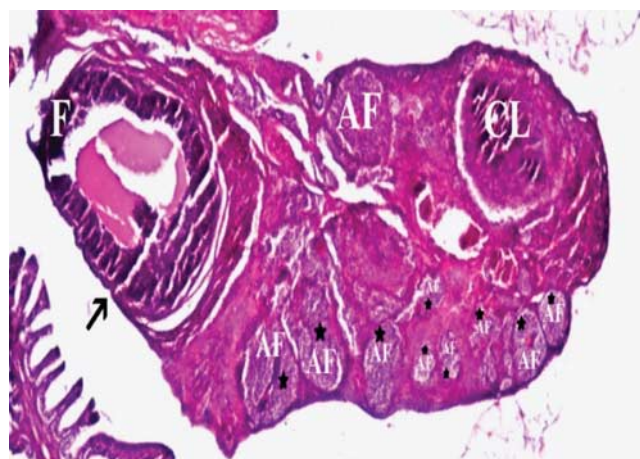
Figure 3

Light photomicrograph of an ovarian section of a control rat (C) showing multiple growing ovarian follicles (F) indicated by the black arrows; the white arrows point to the oocytes. There is one corpus luteum (CL) (H&E × 40).

mechanism that maintains low PRL with high-level gonadal hormones till the evening of proestrus, when the PRL level peaks after the LH surge as described by Nequin *et al.* [14]. When the PRL level became abnormally increased in the SI group, this significant negative correlation became insignificant, indicating a disruption of the regulatory mechanisms that maintain a low secretion of PRL with increased secretion of gonadal hormones till the evening of the proestrus phase.

Reports on estrogen and PRG levels throughout the estrous cycle in the literature have been inconsistent. According to the results of Gomes *et al.* [15], PRG level is lowest during estrus phase and is increased in the evening of diestrus and proestrus phases, reaching a peak value of 30 ng/ml at 23:00 h. This value is much lower than the

Figure 4



Light photomicrograph of an ovarian section of a socially isolated rat showing one growing ovarian follicle (F), indicated by the black arrow, one corpus luteum (CL) and numerous atretic follicles (AF) indicated by the black asterisk (*) (H&E $\times 40$).

PRG level measured in the present study in the C group in the afternoon of proestrus (between 13:00 and 15:00 h). In another study carried out by Nequin *et al.* [14], the PRG level showed a progressive increase in the proestrus phase after 14:00 h, reaching its peak (>84.5 ng/ml) before midnight, which would agree with the PRG level recorded in the present study. In terms of the E2 level recorded in the present study, the time of drawing blood samples was between 13:00 and 15:00 h, which would coincide with the timing of the increase in E2 reported by Gomes *et al.* [15], although the E2 values reported by them during the peak were much lower than the value recorded in the present study. However, Smith *et al.* [18] measured E2 values at 8:00 and 17:00 h of the proestrus phase and were found to be 55 and 57 pg/ml, respectively, which were close to the E2 values recorded in the present study. This variability in the values and timing of changes in the hormone levels throughout the estrous cycle between different studies might be because of the differences in the experimental animal strains and the sensitivity of the biochemical analysis techniques.

The decreased E2 and PRG levels in the SI group were in agreement with the reports of Lachelin and Yen [54], Cameron [50], and Saltzman *et al.* [4]. This decrease in gonadal hormones could be attributed to the observable decrease in the number of the growing ovarian follicles in the SI rat ovary as well as the inhibitory effect of PRL on ovarian steroidogenesis [52]. The significant decrease in E2 in SI rats would entail that the LH surge and the subsequent orchestrated hormonal changes in the proestrus phase would not cascade normally to accomplish successful ovulation. Estrogen is required for the generation of the preovulatory GnRH/LH surge by acting on E₂-sensitive Kiss1 neurons (through ER α) in the anteroventral periventricular nucleus [18]. E₂-sensitive Kiss1 neurons in the arcuate nucleus provide a tonic stimulatory drive to GnRH and are inhibited by estrogen and mediate

the negative feedback effects of sex steroids on GnRH/LH secretion [18]. Moreover, E2 peak acts on lactotrophs and mediates the preovulatory PRL surge in the evening of proestrus [45]. This action is mediated by acting on both types of E2 receptors (α and β) in the preoptic area neurons [55,56].

From the aforementioned data, it can be suggested that social isolation might result in depressed ovarian functions with decreased sex hormone secretion and impaired follicular growth. These effects might be due to local changes in the response of the ovaries to gonadotrophic hormones rather than changes in pituitary gonadotrophic hormones. Further studies are required to elucidate these changes and to determine whether social isolation stress should be considered by gynecologists as a possible cause of ovarian cycle irregularities, unovulation, or infertility in humans.

Acknowledgements

The authors deeply acknowledge Dr Eman K. Habib, lecturer of Anatomy, Faculty of Medicine, Ain Shams University, for her generous effort and kind help in this research.

Conflicts of interest

There are no conflicts of interests.

References

- Weiss RS. Issues in the study of loneliness. In: Peplau D, editor. *Loneliness: a source book of current theory, research, and therapy*. New York: Wiley; 1982. pp. 71–80.
- Sapolsky RM. Social subordination as a marker of hypercortisolism – some unexpected subtleties. *Ann N Y Acad Sci* 1995; 771:626–639.
- Gordon HW. Early environmental stress and biological vulnerability to drug abuse. *Psychoneuroendocrinology* 2002; 27 (1–2): 115–126.
- Saltzman W, Schultz-Darken NJ, Wegner FH, Wittwer DJ, Abbott DH. Suppression of cortisol levels in subordinate female marmosets: reproductive and social contributions. *Horm Behav* 1998; 33:58–74.
- Saltzman W, Severin JM, Schultz-Darken NJ, Abbott DH. Behavioral and social correlates of escape from suppression of ovulation in female common marmosets housed with the natal family. *Am J Primatol* 1997; 41:1–21.
- Hermes GL, Delgado B, Tretiakova M, Cavigelli SA, Krausz T, Conzen SD, McClintock MK. Social isolation dysregulates endocrine and behavioral stress while increasing malignant burden of spontaneous mammary tumors. *Proc Natl Acad Sci USA* 2009; 106:22393–22398.
- Hermes GL, McClintock MK. Isolation and the timing of mammary gland development, gonadarche, and ovarian senescence: implications for mammary tumor burden. *Dev Psychobiol* 2008; 50:353–360.
- Allsworth JE, Clarke J, Peipert JF, Hebert MR, Cooper A, Boardman LA. The influence of stress on the menstrual cycle among newly incarcerated women. *Womens Health Issues* 2007; 17:202–209.
- Ojeda SR, Urbanski HF. The physiology of reproduction. In: Knobil E, Neill JD, editors. *Puberty in the rat*. 2 New York: Raven Press; 1994. pp. 363–409.
- Evans HM, Long JA. *The oestrous cycle in the rat and its associated phenomena*. Berkeley, California: University of California Press; 1922.
- Astwood EB. Changes in weight and water content of the uterus of the normal adult rat. *Am J Physiol* 1939; 126:162–170.
- Hartman CG. Some new observations on the vaginal smear of the rat. *Yale J Biol Med* 1944; 17:99–112.
- Mandl AM. The phases of the estrous cycle in the adult white rat. *J Exp Biol* 1951; 28:576–584.
- Nequin LG, Alvarez J, Schwartz NB. Measurement of serum steroid and gonadotropin levels and uterine and ovarian variables throughout 4 day and 5 day estrous cycles in the rat. *Biol Reprod* 1979; 20:659–670.
- Gomes CM, Rainei C, Ramos de Paula P, Severino GS, Helena CVV, Anselmo-Franci JA, *et al.* Neonatal handling and reproductive function in female rats. *J Endocrinol* 2005; 184:435–445.

- 16 Petersen SL, Ottem EN, Carpenter CD. Direct and indirect regulation of gonadotropin-releasing hormone neurons by estradiol. *Biol Reprod* 2003; 69:1771–1778.
- 17 Shaikh AA. Estrone and estradiol levels in the ovarian venous blood from rats during the estrous cycle and pregnancy. *Biol Reprod* 1971; 5:297–307.
- 18 Smith JT, Popa SM, Clifton DK, Hoffman GE, Steiner RA. Kiss1 neurons in the forebrain as central processors for generating the preovulatory luteinizing hormone surge. *J Neurosci* 2006; 26:6687–6694.
- 19 Gay VL, Midgley AR Jr, Niswender GD. Patterns of gonadotrophin secretion associated with ovulation. *Fed Proc* 1970; 29:1880–1887.
- 20 Parsons B, McGinnis MY, McEwen BS. Sequential inhibition by progesterone: effects on sexual receptivity and associated changes in brain cytosol progesterin binding in the female rat. *Brain Res* 1981; 221:149–160.
- 21 Lydon JP, De Mayo FJ, Funk CR, Mani SK, Hughes AR, Montgomery CA Jr, et al. Mice lacking progesterone receptor exhibit pleiotropic reproductive abnormalities. *Genes Dev* 1995; 9:2266–2278.
- 22 Natraj U, Richards JS. Hormonal regulation, localization, and functional activity of the progesterone receptor in granulosa cells of rat preovulatory follicles. *Endocrinology* 1993; 133:761–769.
- 23 National Research Council (NRC) Committee on Animal Nutrition. *Nutrient requirement of laboratory animals. No. 10.* 3rd revised ed. Washington, DC: National Academy of Science, National Research Council; 1978.
- 24 Reeves PG, Nielsen FH, Fahey GC Jr. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. *J Nutr* 1993; 123:1939–1951.
- 25 Apter SJ, Eriksson CJP. The role of social isolation in the effects of alcohol on corticosterone and testosterone levels of alcohol-preferring and non-preferring rats. *Alcohol and Alcohol* 2006; 41:33–38.
- 26 Marcondes FK, Bianchi FJ, Tanno AP. Determination of the estrous cycle phases of rats: some helpful considerations. *Braz J Biol* 2002; 62 (4A): 609–614.
- 27 Drury RAB, Wallington EA. *Carleton's histological techniques*. 5th ed. New York: Oxford University; 1980. p. 139.
- 28 Armitage P, Berry G. *Statistical methods in medical research*. 2nd ed. Oxford: Blackwell; 1987.
- 29 Figueiredo HF, Dolgas CM, Herman JP. Stress activation of cortex and hippocampus is modulated by sex and stage of estrus. *Endocrinology* 2002; 143:2534–2540.
- 30 Bülbül M, Babygirija R, Cerjak D, Yoshimoto S, Ludwig K, Takahashi T. Impaired adaptation of gastrointestinal motility following chronic stress in maternally separated rats. *Am J Physiol Gastrointest Liver Physiol* 2012; 302:G702–G711.
- 31 Castagliuolo I, LaMont JT, Qiu B, Fleming SM, Bhaskar KR, Nikulasson ST, et al. Acute stress causes mucin release from rat colon: role of corticotropin releasing factor and mast cells. *Am J Physiol Gastrointest Liver Physiol* 1996; 271 (34–5): G884–G892.
- 32 Dohi K, Kraemer WJ, Mastro AM. Exercise increases prolactin-receptor expression on human lymphocytes. *J Appl Physiol* 2003; 94:518–524.
- 33 Sobrinho LG. Prolactin, psychological stress and environment in humans: adaptation and maladaptation. *Pituitary* 2003; 6:35–39.
- 34 Insana SP, Wilson JH. Social buffering in rats: prolactin attenuation of active interaction. *Psychol Rep* 2008; 103:77–87.
- 35 Ranabir S, Reetu K. Stress and hormones. *Indian J Endocrinol Metab* 2011; 15:18–22.
- 36 Seggie JA, Brown GM. Stress response patterns of plasma corticosterone, prolactin, and growth hormone in the rat, following handling or exposure to novel environment. *Can J Physiol Pharmacol* 1975; 53:629–637.
- 37 Jahn GA, Deis RP. Stress-induced prolactin release in female, male and androgenized rats: influence of progesterone treatment. *J Endocrinol* 1986; 110:423–428.
- 38 Meyerhoff JL, Oleshansky MA, Mougey EH. Psychologic stress increases plasma levels of prolactin, cortisol, and POMC-derived peptides in man. *Psychosom Med* 1988; 50:295–303.
- 39 Voigt KH, Frank D, Duker E. Dopamine-inhibited release of prolactin and intermediate lobe-POMC-peptides: different modulation by opioids. *Life Sci* 1983; 33 (Suppl 1): 507–510.
- 40 Akema T. Permissive role of corticotropin-releasing factor in the acute stress-induced prolactin release in female rats. *Neurosci Lett* 1995; 198:146–148.
- 41 Polston EK, Erskine MS. Excitotoxic lesions of the medial amygdala differentially disrupt prolactin secretory responses in cycling and mated female rats. *J Neuroendocrinol* 2001; 13:13–21.
- 42 Shennan DB. Regulation of water and solute transport across mammalian plasma cell membranes by prolactin. *J Dairy Res* 1994; 61:155–166.
- 43 Struman I, Bentzien F, Lee H, Mainfroid V, D'Angelo G, Goffin V, et al. Opposing actions of intact and N-terminal fragments of the human prolactin/growth hormone family members on angiogenesis: an efficient mechanism for the regulation of angiogenesis. *Proc Natl Acad Sci USA* 1999; 96:1246–1251.
- 44 Torner L, Karg S, Blume A, Kandasamy M, Kuhn HG, Winkler J, et al. Prolactin prevents chronic stress-induced decrease of adult hippocampal neurogenesis and promotes neuronal fate. *J Neurosci* 2009; 29:1826–1833.
- 45 Smith MS, Freeman ME, Neill JD. The control of progesterone secretion during the estrous cycle and early pseudopregnancy in the rat: prolactin, gonadotropin and steroid levels associated with rescue of the corpus luteum of pseudopregnancy. *Endocrinology* 1975; 96:219–226.
- 46 Neill JD, Freeman ME, Tillson SA. Control of the proestrus surge of prolactin and luteinizing hormone secretion by estrogens in the rat. *Endocrinology* 1971; 89:1448–1453.
- 47 Neill JD, Smith MS. Pituitary-ovarian interrelationships in the rat. In: James VHT, Martini L, editors. *Curr Top Exp Endocrinol*. New York: Academic; 1974. pp. 73–106.
- 48 Yen SH, Pan JT. Progesterone advances the diurnal rhythm of tuberoinfundibular dopaminergic neuronal activity and the prolactin surge in ovariectomized, estrogen-primed rats and in intact proestrous rats. *Endocrinology* 1998; 139:1602–1609.
- 49 Freeman ME, Kanyicska B, Lerant A, Nagy G. Prolactin: structure, function, and regulation of secretion. *Physiol Rev* 2000; 80:1523–1631.
- 50 Cameron JL. Stress and behaviorally induced reproductive dysfunction in primates. *Semin Reprod Endocrinol* 1997; 15:37–45.
- 51 Larsen JL, Bhanu A, Odell WD. Prolactin inhibition of pregnant mare's serum stimulated follicle development in the rat ovary. *Endocr Res* 1990; 16:449–459.
- 52 Villanueva LA, Méndez I, Ampuero S, Larrea F. The prolactin inhibition of follicle-stimulating hormone-induced aromatase activity in cultured rat granulosa cells is in part tyrosine kinase and protein kinase-C dependent. *Mol Hum Reprod* 1996; 2:725–731.
- 53 Shimizu T, Oshima I, Ozawa M, Takahashi S, Tajima A, Shiota M, et al. Heat stress diminishes gonadotropin receptor expression and enhances susceptibility to apoptosis of rat granulosa cells. *Reproduction* 2005; 129:463–472.
- 54 Lachelin GCL, Yen SSC. Hypothalamic chronic anovulation. *Am J Obstet Gynecol* 1978; 130:825–831.
- 55 Pan JT, Gala RR. Central nervous system regions involved in the estrogen-induced afternoon prolactin surge. II. Implantation studies. *Endocrinology* 1985; 117:388–395.
- 56 Li HY, Blaustein JD, De Vries GJ, Wade GN. Estrogen-receptor immunoreactivity in hamster brain: preoptic area, hypothalamus and amygdala. *Brain Res* 1993; 631:304–312.