PSYCHOGENIC PREGNANCY BLOCKS

HORMONAL CORRELATES OF PSYCHOGENIC PREGNANCY BLOCKS

By

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ABSTRACT

Various psychological stressors have adverse effects upon the first period of pregnancy in humans and other mammals, however the underlying hormonal mechanisms are not established. A review of previous research suggests that the hormones normally associated with stress, the adrenal corticosteroids, are not important in the mediation of this effect. This thesis examined the roles of the primary gonadal hormones, progesterone and estrogen, in stress-induced pregnancy blocks.

Experiment 1 established a restraint stress procedure as a reliable paradigm for the examination of stress effects in pregnancy in an animal model. Restrained females produced significantly fewer litters than did controls.

Experiments 2 to 4 were designed to evaluate the contribution of progesterone to stress-induced pregnancy blocks. Experiment 2 replicated the restraint effect in two strains of mice, and demonstrated that daily administration of 500 ug progesterone will reverse this effect in HS but not C57 mice. Experiment 3 demonstrated that exposure to a predator will also block pregnancy in C57 mice, although this effect was not consistent for the HS strain. This pregnancy block in C57 mice can be counteracted with concomitant progesterone administration. Experiment 4 showed that metyrapone, a compound which prevents the conversion of progesterone to corticosterone, was also partially effective in maintaining pregnancy under stressful circumstances.

Experiments 5 to 7 were designed to assess the contribution of estrogen in pregnancy blocks caused by stress. In Experiment 5, the dose-response curve for unmodified 17β-estradiol was examined. Daily dosages of 0.333 υg and greater completely blocked pregnancy, that of 0.111 υg did so in the majority of females, while lesser dosages had little apparent affect. For comparison, the dose-response curve for estradiol 17β-benzoate was determined in Experiment 6. Results were very similar to those for 17β-estradiol in that pregnancy was completely blocked at daily dosages of 0.333 υg and greater, and there were only two completed pregnancies at a daily dosages of 0.111 υg, while females receiving lower doses were indistinguishable from controls. Given that estrogen is such a potent blocker of pregnancy, it is conceivable that if stress resulted in even minute increases in the endogenous estrogen levels, that pregnancy would fail. Experiment 7 demonstrated that daily administration of an antibody to estrogen will reverse a stress-induced pregnancy block.

Experiment 8 shows direct measures, obtained via radioimmunoassay, of corticosterone, progesterone and estradiol in pregnant animals who were stressed or not. All three of these steroids were significantly elevated in the stressed animals.

These results of this thesis suggest that estrogenic action may mediate stress-induced pregnancy blocks, and that estrogen may serve as a "stress hormone".

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CHAPTER ONE
INTRODUCTION

The Stress Concept

It has been known for many years that exposure to various challenging environmental events will cause a stereotyped physiological response in mammals. This concept of "stress" was first formally described by Walter Cannon in 1914. In a paper outlining the interactions between emotions and physiology, he detailed psychoendocrine relationships in which "great emotional stress" or "times of need or stress" could disturb normal bodily functioning (Cannon, 1914). Later, Cannon imported the metallurgical engineering concept of stress to physiology. To engineers, stress is an internal force capable of distorting atomic structure, and generated within a solid body in response to the application of an external load (Smith, 1987). Loosely applied to a physiological system, Cannon conceived of stress as an external force that, when applied, could disrupt homeostatic efficiency. This effect occurred after physical stimuli, including low temperatures and lack of oxygen, as well as emotional stimuli such as worry and scholastic demands. Cannon also described the actions of the sympatho-adrenal system which functions to return the body to homeostasis (Cannon, 1935).

By 1936, the stress concept had been operationalized in physiological terms by Hans Selye. He had found that a wide variety of noxious stimuli such as injections of small amounts of toxic fluids, temperature changes, x-rays and intense nervous excitement all produced a stereotyped triad of morphological changes: 1) enlargement of the adrenal cortex, 2) lymphatic system atrophy, and 3) stomach and duodenal ulcers. Selye suggested that these changes comprise a "General Adaptation Syndrome" (G.A.S.), and were incurred after the prolonged endocrine response to external agents acting upon the body (Selye, 1973).

The Stress Response

Demanding external events imposed on an organism result in a number of physiological consequences. Such events include confrontation with a predator, or exposure to extremes in temperature or altitude. The presence of the particular challenging stimulus is relayed to the cerebral cortex via the afferent sensory nerves. Once the nervous system has perceived and integrated the presence of the external challenge, two primary physiological systems are activated in order to deal with the challenge: the sympathetic nervous system and the hypothalamic-pituitary axis (Selye, 1936).

The consequences of sympathetic activation include increased heart rate and blood pressure, and increased sweat secretion, as well as respiratory changes. The adrenal medulla is also stimulated to secrete epinephrine which serves to amplify these sympathetic responses, as well

as to mobilize glucose stores from the liver in order to make available the energy required to deal with the demand. Furthermore, bodily vegetative processes that are under parasympathetic mediation, such as gastric secretion and intestinal motility, are depressed (Cannon, 1929).

Cortical activation in response to a stressor results in excitation of the hypothalamus via hypothalamo-cortical neuronal pathways. This causes the immediate secretion of corticotropin-releasing factor (CRF) from the hypothalamus into the primary capillary plexus of the hypothalamic-hypophyseal portal system. CRF in turn stimulates the anterior pituitary to secrete large amounts of adrenocorticotropic hormone (ACTH). These rapid (less than 30 seconds) events comprise the first stage of physiological response to a challenging stimulus. This corresponds to the first temporal phase of the G.A.S. which Selye called the Alarm Reaction. The ACTH released then travels through the bloodstream to the adrenal gland where it activates the adrenal cortex to synthesize glucocorticoids. This stress-induced increase of glucocorticoid secretion continues for the duration of the external stimulus and constitutes the Phase of Resistance. However, if the stimulus persists for a long period of time, the adrenal cortex will fail to sustain this high level of glucocorticoid production resulting in adverse physiological effects. This last stage in the GAS describes the effects of chronic stress and is called the Phase of Exhaustion (Selye, 1956).

In addition, the whole hormonal stress response is self-regulated via both short and long negative feedback loops. The first short loop involves the transmission of corticoid levels in the adrenal cortices back to the pituitary. High levels of these glucocorticoids will

serve to inhibit ACTH production. The second loop feeds back from the pituitary to the hypothalamus. Large quantities of ACTH present in the pituitary will decrease the amount of CRF being released from the hypothalamus. The long negative feedback loop extends from the peripheral blood vessels directly back to the hypothalamus. Again, large quantities of glucocorticoids present in the peripheral blood flow will result in inhibition of CRF release. It should be noted that the onset of a second stressful stimulus will "break through" these negative feedback loops to allow for adequate physiological compensation for the stressful state (Axelrod & Reisine, 1984; Davidson, Jones & Levine, 1968).

Support for the stereotypical biochemical concomitants of the physiological response to stressors has been demonstrated experimentally utilizing a variety of external stimuli in a number of animal species. Included are shock in rats (Friedman, Ader, Grota & Larson, 1967), handling in monkeys (Elvidge, Challis, Robinson, Roper & Thorburn, 1976), restraint in mice (Barlow, McElhatton, Morrison, and Sullivan, 1974; Barlow, Morrison & Sullivan, 1975) and gerbils (Fenske, 1986), social dominance in baboons (Sapolsky & Ray, 1989), conditioned emotional response in monkeys (Mason, Mangan, Brady, Conrad & Rioch, 1961), repeated venepunctures in rabbits (Fenske, Grospietsch & Konig, 1981a; Fenske et al., 1981b), and learned avoidance behaviours in rats (Levin & Brush, 1967).

The Acute/Chronic Stress Distinction

Ordinarily, four types of stressful events are recognized and are distinguished on the basis of duration. First, researchers discuss acute or time limited stressful events such as experienceing a venepuncture. Second, there are "stress event sequences" which involve a number of these acute events that occur over an extended period. Chronic intermittent stressors involve demanding situations which reoccur unpredictably over time. Finally, there is the chronic stress situation which involves the experience of a constant unescapable stressor over a long period (Cohen, 1983).

It is also important to distinguish between an unavoidable stressor and one which can be eliminated or controlled. Studies have shown that if animals can predict the onset and duration of electric shocks, or can perform responses that will allow them to control or escape the shocks, then the physiological effects of stress will be lessened as compared to yolked controls (Levine, 1971). Consequently, when the effects of stress on physiology are being addressed, it is generally the effects of a chronic unavoidable stressor that are in question.

It is now generally accepted that Selye's original proposition of a nonspecific response to a stressor is not entirely accurate. Indeed, it has become apparent in recent years that the physiological response is dependent on a host of variables including the nature of the stressor

employed, its intensity, its frequency (Terman, Shavit, Lewis, Cannon & Liebskind, 1984), and the species of organism at which it is directed (Gibbs, 1984). For consistency and clarification, the operational definition of stress to be utilized in this thesis specifies that the previously described stereotypical biochemical response to an external disruption is induced in response to an inescapable challenge.

Physiological Consequences of Stress

The purpose of the stress reaction is an adaptive one. The physiological changes that occur in response to a stimulus allow the organism to deal with a situation which it could not address under resting conditions. Thus, this mobilization for "fight or flight" maximizes the probability of adaptive responding under demanding circumstances, and increases the chances of organismic survival (Cannon, 1929).

Stimuli which evoke these physiological reactions, referred to as stressors, can also have varied detrimental effects on many aspects of the organism's well-being. Indeed, if the adrenal gland produces a surplus of glucocorticoids in order to allow the body to cope with stressors, then it is likely that an abnormal hormonal excess such as that experienced under chronic stress conditions would have deleterious consequences. Selye discussed a variety of "diseases of adaptation" caused by stress-induced alterations of endogenous hormonal homeostasis.

For example, injections of deoxycorticosterone (DOC), in addition to a salt enriched diet, cause a syndrome analogous to Bright's

Disease, or nephrosclerosis, in a number of animal species. This effect is also produced by pituitary extracts other than ACTH, providing the animals are in possession of intact adrenal glands (Selye, 1956). Further support for adrenal activity as the etiology of this disorder is demonstrated in human patients who show increased levels of adrenal corticoids in their urine. Furthermore, adrenalectomy abolishes their nephrosclerotic symptoms (Selye, 1956).

Other maladies such as rheumatoid arthritis and cardiovascular disorders have also been produced in animals by the administration of DOC and other adrenal compounds such as aldosterone, and have been alleviated in humans by adrenalectomy (Selye, 1956).

The adverse physiological effects of adrenal hormones associated with stressors have been demonstrated. The role of stressors themselves in pathogenesis are also being elucidated. Selye suggested that the gastrointestinal system was particularly sensitive to the effects of stressors. This premise was based on the observation that loss of appetite and irritations of the digestive tract, such as nausea and diarrhea, are among the first physiological concomitants of psychological stressors such as examination stress. Gastrointestinal malaise in the extreme form consists of gastric and duodenal ulcers which are also produced by the application of stressors in both man and animal models. Furthermore, adrenalectomy prevents stress-induced ulceration in rats. (Selye, 1956; 1973). Since Selye's pioneering work, the demonstration that stressful life experiences can lead to the development of gastric and duodenal ulceration has been replicated often (Weiss, 1971).

Stress has also been implicated in the etiologies of a number of other physical disorders. The relationship of aversive life events to coronary heart disease (Glass, 1977; Jenkins, 1976; Theorell, 1981) and hypertension (Diamond, 1982) are well known. Other less known illnesses in which stress is considered to be an exacerbating factor include cancer (Shekelle, Raynor, Ostfeld, Garron, Bieliauskas, Liu, Maliza & Oglesby, 1981; Sklar & Anisman, 1980), diabetes (Cox, Taylor, Nowacek, Holley-Wilcox, Pohl & Guthrow, 1984), and mononucleosis (Kasl, Evans & Neiderman, 1979). Due to the effects of stressors on the noradrenergic system, stress has also been implicated in the precipitation of some mental disorders such as schizophrenia (Smith, 1987), mania (Depue & Monroe, 1979), and particularly depression (see Anisman & Zacharko, 1982 for a review).

CHAPTER TWO

The Effects of Stress on Reproduction

The finding that stressors have such a wide range of adverse physiological consequences suggests that any physiological system may be vulnerable to stress effects, but the resultant pathology will be dependent on the nature of the affected system (Anisman & Zacharko, 1982). Given the dual role of the pituitary in modulating both adrenal and gonadal hormone release, and the interdependence of these systems, it is surprising that little attention has been directed at the effects of stress on reproductive function (Reichlin, Abplanalp, Labrum, Schwarz, Sommer & Taymor, 1979). Furthermore, it has been shown that pregnant animals, as compared to nonpregnant controls, exhibit a much greater corticosterone response to stress. In one experiment, the timing of the corticosterone surge was the same for both groups, but the pregnant group experienced corticosterone levels 10 times that of the nonpregnant animals. It is reasonable to propose that such high circulating levels of corticosterone can be detrimental to pregnancy outcome (Barlow et al., 1975). What impact does stress have on the various hormonally mediated reproductive parameters?

Effects of Stressors on Human Conception and Pregnancy

It has been shown that the stress of rigorous athletic activity such as long distance running may lead to cessation of menstruation (Dunbar, 1985). Given the strenuous direct wear on the body of this activity, any effects on reproductive function may be more related to the physical aspects of this stressor rather than to any emotional challenges. However, the underlying hormonal changes resultant from stress are consistent regardless of whether the stressor has physical or psychogenic origins. Other studies have suggested that more psychological stressors such as shiftwork (Chenier, undated), university examinations and intense noise can lead to disruptions of normal menstrual cycling (Fremont-Smith & Meigs, 1948), and that emotional distress can lead to anovulation (Rutherford et al., 1961). One author suggests that amenorrhea can also be caused by various types of psychological trauma such as living away from home for the first time, or being incarcerated in jail or a prison camp (Hubble, 1963).

The idea that psychological stress experienced during pregnancy could be linked to obstetric complications has been alluded to in folklore for centuries. One popular belief suggested that exposure to an emotionally arousing object during pregnancy resulted in assimilation of the appearance of the object by the fetus. Thus, if a pregnant women was scared by a runaway horse, her child would be expected to take on equine features and behaviours (Williamson, 1890 cited in Istvan, 1986). A variant of this belief in "maternal impressions" involved a broader conception of the effects of psychological excitation during pregnancy.

Researchers now focus on this more general idea that emotional excitement experienced in pregnancy can lead to a variety of nonspecific responses in the fetus or in the course of gestation. Two areas of investigation have emerged: the question of whether stress experienced during pregnancy can have detrimental effects on the course and viability of pregnancy, and effects on the organic and behavioural features of the offspring.

The earliest studies of this nature attempted to relate stress with "malformations" in the offspring including mental retardation, epilepsy and cerebral palsy (CP). This work was complicated by the fact that the investigators retrospectively examined the birth records of patients diagnosed with CP or mental retardation, and that they considered "stress" in terms of complications experienced during pregnancy. Results indicated that afflicted children had been exposed to more problems during delivery, such as maternal bleeding or infection or abnormal birth positions, than a control group of normal children (Lilienfeld & Parkhurst, 1951; Lilienfeld & Pasamanick, 1955; Pasamanick & Lilienfeld, 1955). This notwithstanding, it is difficult to take these results to suggest that psychological stress contributed to abnormal fetal outcome. The definition of stress used was " the presence of pregnancy complications". These complications may not have been stress related at all, and another variable may have better explained these results.

Table 1 chronologically summarizes the results found to date on the effects of stress experienced during pregnancy. A critical analysis of this group of studies as a whole will follow the review. The first

Table 1

Table Summarizing Studies of the Relationship Between Maternal Stress and Obstetric Outcome

Authors	Stress Measures	Outcome
Cramond, 1954	anxiety measured by retrospective interview, MAS, MMPI	no relationship of anxiety measures to labour experience
Scott & Thompson, (1956)	Neuroticism (MMQ), global stability rating in 3rd trimester	women rated as unstable had fewer delivery complications
Stott (1958)	review of case studies	number of "shocks" experienced during pregnancy correlated with probability of abnormality
Davids, DeVault, & Talmadge (1961)	MAS in 3rd trimester	"abnormal births" associated with high anxiety score
Grimm (1961)	TAT at 5 points over gestation period	"tension" associated with weight gain, length of 2nd stage labour
Hetzel, Bruer, & Poidevin (1961)	retrospective interview of stress life events during gestation	high life stress associated with excessive vomiting & toxemia
Davids & DeVault, (1962)	MAS, TAT, self rating, rating of other, global psychologist's rating	high anxiety scores on all measures but self rating related to "abnormal birth"
Kapp, Hornstein, & Graham, (1963)	retrospective interview of "habitual anxieties"	abnormal labour associated with higher anxiety

Table 1 Continued

Authors	Stress Measures	Outcome
McDonald & Christakos (1963)	MAS during 3rd trimester	"abnormal pregnancy" associated with high anxiety
Gunter (1963)	TAT, life changes as assessed by interview post partum	positive association between life changes and prematurity but not TAT
McDonald, Gynther, Christakos (1963)	IPAT during 3rd trimester	"abnormal pregnancy" and long labour associated with high anxiety
McDonald & Parham, (1964)	MAS during 3rd trimester	no relationship to any measures of pregnancy
McDonald (1965)	MAS, MMPI during 3rd trimester	high MAS score related to abnormalities; no relation for MMPI
Grimm & Venet (1966)	Neuroticism, anxiety assessed in 1st trimester and situational stresses in 3rd trimester	no relationship between obstetric outcome and any psychological variables
Edwards & Jones (1970)	STAI state scale weekly until delivery, trait scale in 3rd trimester	no relationship between obstetric outcome and STAI
Nuckolls, Cassel, & Kaplan (1972)	SAQ in 2nd trimester, SRE in 3rd trimester	high life changes plus low social assets related to obstetric problems
Burstein, Kinch, & Stern (1974)	MAS, pregnancy anxiety scale sometime during pregnancy	no relationship between obstetric outcome and these measures

Table 1 Continued

Authors	Stress Measures	Outcome
Gorsuch & Key (1974)	SRE, trait scale from STAI in 1st or 2nd trimester, state monthly thereafter, SRE after delivery	high anxiety, high life changes related to pregnancy abnormalities
Williams et al., (1975)	SRE given post partum	no relationship to prematurity
Jones (1978)	MAS, SRE, MMPI within 2 weeks of delivery	no association of these measures with obstetric outcome
Lederman et al., (1978)	STAI at onset of labour	no association with length of labour
Crandon, (1979a)	IPAT in 3rd trimester	high anxiety related to obstetric complications
Crandon, (1979b)	IPAT in 3rd trimester	high anxiety related to low Apgar scors
Falorni et al., (1979)	IPAT, interview of "troubles" completed after childbirth	high anxiety related to longer labour
Lederman et al., (1979)	STAI- state scale during labour; trait after birth	state anxiety but not trait related to long labour
Morcos & Funke-Ferber (1979)	MAS, Pitts Depression Scale in 1st & 3rd trimesters	no association of these measures with obstetric outcome
Newton et al., (1979)	LEI given post partum	positive relation to prematurity
Standley et al., (1979)	anxiety assessed by interview in last week of pregnancy	anxiety about impending birth and childcare correlated with poor motor maturity
Beck et al., (1980)	STAI trait scale in 3rd trimester, state scale before delivery	no association of these measures with obstetric outcome

Table 1 Continued

Authors	Stress Measures	Outcome
Areskog et al., (1983)	"fear of delivery" assessed by interview prior to due date	high fear related to labour complications
Norbeck & Tilden (1983)	STAI, "emotional disequilibrium", other psychometric tests in 2nd or 3rd trimester	high life stress and "emotional disequilibrium" related to obstetric problems
Newton & Hunt (1984)	STAI; LEI once each trimester	LEI but not STAI associated with prematurity and low birth weight
Smilkstein et al., (1984)	SRE, biomedical risk data for 1 year before pregnancy before delivery, SRE 2-3 days postpartum	no association of these measures with obstetric outcome
Rizzardo et al., (1985)	PIRLE, MMQ during 6th month gestation	anxiety but not life events related to obstetric problems

Note: MAS = Taylor Manifest Anxiety Scale; MMPI = Minnesota Multiphasic Personality Inventory; MMQ = Maudsley Medical Questionnaire; IPAT = Institute for Personality and Ability Testing Anxiety Self-Analysis Form; STAI = State-Trait Anxiety Inventory; SAQ = Social Assets Questionnaire; SRE = Holmes-Rahe Schedule of Recent Events; LEI = Life Events Inventory

controlled study on record in this area was done by Cramond in 1954. In a retrospective interview performed shortly after childbirth, anxiety during pregnancy was assessed using the Minnesota Multiphasic Personality Inventory (MMPI) and the Taylor Manifest Anxiety Scale (MAS). Scores on these tests were compared to an evaluation of the course of labour. Surprisingly, results indicated that mothers who had experienced complications such as prolonged labour actually scored lower on the MAS than those having normal deliveries. Because the difficult delivery group also reported a higher incidence of ulcers, Cramond (1954) concluded that these women must be internalizing their psychological distress. Support for this notion came from another British study (Scott & Thompson, 1956) which assessed "neuroticism" using the Maudsley Medical Questionnaire (MMQ). These investigators found that women low in neuroticism, the "emotionally stable" group, experienced fewer delivery complications than those women assessed as being "unstable". However, one study reported that there was no relationship between neurotic anxiety and obstetric outcome (Grimm & Venet, 1966).

In 1958, Stott published a review article in which he reported a number of case studies of women who had experienced at least one stressor during the course of pregnancy. Mothers who had been exposed to many stressors such as car accidents, a bombing raid in World War 2, or intense anxiety or grief also gave birth to a larger number of mentally deficient children than those experiencing fewer or no stressful events.

A host of studies performed throughout the 1960s and 1970s confirmed, for the most part, that there may be some effect of

psychological stress on pregnancy outcome. Interviews performed retrospectively in which mothers were simply asked about stress experienced during pregnancy revealed an association between high anxiety prenatally and a variety of obstetric complications (Hetzel, Bruer & Poidevin, 1961; Kapp, Hornstein & Graham, 1963; Standley, Soule & Copans, 1979). Similarly, one study reported a positive correlation between a woman's "fear of giving birth" and problems experienced over the course of labour (Areskog, Uddenburg & Kjessler, 1983)

Other investigators have utilized more standardized interview methods. Davids, DeVault & Talmadge (1961) administered the MAS during the third trimester of pregnancy and found that high anxiety scores were positively correlated with delivery abnormalities. These results were replicated by the same team (Davids & DeVault, 1962) and later supported by another group of investigators who quantified the course of pregnancy as either normal or abnormal based on the length of labour and the presence of any other obstetric complications. When correlated with MAS score obtained in third trimester, there was a positive significant relationship between high anxiety and obstetric abnormality (McDonald, 1968; McDonald & Christakos, 1963).

Conversely, other investigators who also used the MAS to ascertain whether prenatal stress is associated with obstetric complications failed to find any such relationship. Burstein, Kinch & Stern (1974) administered the MAS, as well as the Pregnancy Anxiety Scale, at some point during the course of gestation. No relationship was found between either of these psychological measures and any aspect of pregnancy outcome. Similarly, no association was found between obstetric outcome

and the anxiety when the MAS was administered at a point in both the first and third trimesters (Morcos & Funke-Ferber, 1979) or in the third trimester alone (Jones, 1978; McDonald & Parham, 1964)).

Other measures of psychological stress have also been utilized. The Thematic Apperception Test (TAT) is a projective test incorporating subjective scoring of subjects' interpretations of a number of ambiguous pictures. Grimm (1961) reported that "psychological tension" reflected by TAT responses was associated with total weight gain over pregnancy and longer periods of labour. Similarly, Davids & DeVault (1962) also reported that anxiety as indicated by the TAT was associated with delivery complications, however another failed to find a significant relationship between TAT anxiety and prematurity (Gunter, 1963).

Another psychometric tool used in this area is the Institute for Personality and Abilities Testing Anxiety Self-Analysis Form (IPAT). One group of researchers reported that high anxiety as measured by this test in the third trimester was associated with delivery complications. This was supported by Crandon (1979a; 1979b), and in one study where the IPAT was administered retrospectively two weeks after childbirth (Falorni, Fornsarig & Stefanile, 1979).

Anxiety as measured by the Holmes-Rahe Schedule of Recent Experience (SRE), has also been related to abnormalities over the course of pregnancy (Gorsuch & Key, 1974; Nuckolls, Cassel & Kaplan, 1972), however other investigators have failed to obtain an association to either gestational abnormalities (Smilkstein, Helsper-Lucas, Ashworth, Montano & Pagel, 1984) or prematurity (Williams, Williams, Griswald & Holmes, 1975). A similar tool, the Paykel Interview of Recent Life Events,

did not show any relationship of stressful experiences to obstetric complications (Rizzardo, Magni, Abdreoli, Merlin, Andreoli, Fabbris, Martinotti & Cosentino, 1985). These investigators did report an association of free anxiety, as assessed by the Middlesex Hospital Questionnaire, and problems during pregnancy (Rizzardo et al., 1985).

Psychosocial stress has also been measured using the Life Events Inventory (LEI). In application with pregnant women, studies report a positive relationship between the experience of stressful life events during pregnancy and both prematurity (Newton, Webster, Binu, Maskrey & Phillips, 1979; Newton & Hunt, 1984) and low birth weight (Newton & Hunt, 1984).

The most popular psychometric test used to determine anxiety or psychological stress is the Stait-Trait Anxiety Inventory (STAI). Some investigators using this device reported an association between both state and trait anxiety scales and abnormalities during gestation or in pregnancy outcome (Norbeck & Tilden, 1983), while others reported no relationship (Beck, Seigle, Davidson, Kormeier, Britenstein & Hall, 1980; Edwards & Jones, 1970; Newton et al., 1979). One investigator measured trait anxiety shortly post partum, and state anxiety at the onset of each stage of labour. Results indicated that whereas the more general measure of state anxiety was not related to any aspects of pregnancy outcome, state anxiety was positively correlated with the length of labour (Lederman, Lederman, Work, & McCann, 1979).

Summary and Conclusions

Of the 33 studies reviewed herein, one third (11) reported no relationship between psychological stressors experienced over gestation and pregnancy outcome. The remaining studies are fraught with methodological problems that limit the validity of their conclusions.

Most of these studies (Beck et al., 1980; Burstein et al., 1974; Crandon, 1979a; 1979b; Davids et al., 1961; Davids & DeVault, 1962; Edwards & Jones, 1970; Grimm & Venet, 1966; Gorsuch & Key, 1974; Hetzel, et al., 1961; Marcos & Funke-Ferber, 1979; McDonald, 1965; McDonald & Christakos, 1963; McDonald & Parham, 1964; McDonald et al., 1963; Norbeck & Tilden, 1983; Nuckleby et al., 1972; Scott & Thompson, 1956) relied almost exclusively on self report measures which may be biased, especially if done retrospectively as many were (Cramond, 1954; Falorni et al., 1979; Jones, 1978; Kapp et al., 1963; Smilkstein et al., 1984; Standley et al., 1978; Stott, 1958). It is known that self-report measures can be subject to either inflation or deflation of actual experience and thus may not be accurate (Azrin, Holz & Goldiamond, 1961). Only one of these studies attempted to interview significant others, such as spouses or other family members, in order to obtain convergent data with those of the mother (Davids & DeVault, 1962). Furthermore, most of these studies (Beck et al., 1980; Burstein et al., 1974; Cramond, 1954; Crandon, 1979a; 1979b; Davids et al., 1961; Davids & DeVault, 1962; Edwards & Jones, 1970; Falorni et al., 1979; Grimm & Venet, 1966; Gorsuch & Key, 1974; Hetzel, et al., 1961; Jones, 1978; Kapp et al., 1963; Marcos & Funke-Ferber, 1979;

McDonald, 1965; McDonald & Christakos, 1963; McDonald & Parham, 1964; McDonald et al., 1963; Norbeck & Tilden, 1983; Nuckolls et al., 1972; Scott & Thompson, 1956; Smilkstein et al., 1984; Standley et al., 1978; Stott, 1958) neglect the inclusion of a control population for comparison purposes, and some base their conclusions on extremely small sample sizes (Gunter, 1963; Kapp et al., 1963; Lederman et al., 1978; Stott, 1958). It is also difficult to compare across studies due to the differences in experimental design, as well as in anxiety or stress measures, pregnancy measures, and populations sampled. Furthermore, it is possible that there are factors associated with high measures of maternal anxiety, such as low socioeconomic status or tobacco use, that may better account for any abnormalities observed in pregnancy (Istvan, 1986). Also, a recent study has shown that there is much variance in both the amount and type of psychological stress experienced by mothers during the three trimesters of pregnancy (Arizmendi & Affonso, 1987). Consequently, critical reviews of the human literature regarding the effects of prenatal stress and anxiety on birth outcome conclude that there is only weak evidence suggesting a link. However, this is primarily due to the limitations of the existing studies, and better experimental design and methodology may help to elucidate the relationship (Istvan, 1986; Levin & DeFrank, 1988).

Animal Models of Stress Effects on Reproduction

It is apparent that the human studies of stress effects on pregnancy are largely inconclusive due to methodological problems and

uncontrollable variance. Consequently, as in virtually all areas of biomedical research, investigators have turned to animal models of stress paradigms. Numerous stressors experienced at different times throughout the course of pregnancy will lead to embryonic loss in a number of animal species. This response is primarily adaptive in that it is logical that an organism should postpone reproduction until more optimal environmental circumstances are present. Much evidence suggests that stressful stimuli administered during the first third of pregnancy can impair implantation of fertilized ova or provoke abortion or resorption of fetuses (Hsu, 1948; MacFarlane, Pennycuik, and Thrift, 1957). A number of stressor paradigms have been established to study stress effects on reproductive parameters. Although these stimuli vary with respect to method and time course, all have been shown to elicit the physiological responses characteristic of the stress response. Table 2 presents the findings of studies examining the effects of various stressors on pregnancy.

Overcrowding

Early laboratory experiments mimicked environmental observations (see Christian, Lloyd & Davis, 1965 for a review) that overpopulation could lead to a decline in reproductive rate. Calhoun (1949) housed a population of wild Norway rats in a small enclosure for a long period of time and observed a decrease in the number of pups born during this time. Similarly, Christian and LeMunyan (1958) achieved experimental crowding by housing mixed sex groups of 40 mice in a cage

Table 2

Table Summarizing the Results of Animal Experiments on the Effects of Prenatal Stress on Pregnancy Outcome

Authors	Stress Paradigm	Outcome
Calhoun (1949)	rat population housed in small pen	decreased number of pups especially in socially subordinate females
Christian & LeMunyan, 1958	large group of rats housed in small cage for 6 weeks	no young born during crowded period
Hsu (1948)	exposure of rats to 39°C	decreased number of term pregnancies
Runner (1955)	weighing or injecting vehicle daily in mice	decreased number live young born
MacFarlane, Pennycuick & Thrift (1957)	rats housed at 35°C	increased resorption rate
Christian & LeMunjan (1958)	overcrowding in rats	fewer litters born to crowded females
Zondek & Tamari (1958)	intermittent noise in rats	decreased number of offspring born
Helmreich (1960)	overcrowding in rats	increased in utero resoption in grouped females
Hartel & Hartel (1960)	3 hours restraint day 9; 4 hours days 10 to 12 in rats	increased number resorbed embryos
Hartel & Hartel (1960)	6 hours intermittent bells and flashing lights in rats	no effect on number resorbed embryos
Eleftheriou, Bronson & Zarrow (1962)	exposure of mice to isolation, other mice, novel or smaller cage,	decreased incidence of pregnancy

Table 2 Continued

Authors	Stress Paradigm	Outcome
Stott & Williams, (1962)	cattle bred during high temperature season	increased embryonic mortality
Weir & DeFries (1963)	forced swimming, exposure to heat, light and noise in mice	decreased fertility rate
Zondek & Tamari (1967)	exposure to intermittent noise during days 4-6, 7-9, or 8-10 in rats	decreased number of offspring especially during last period
Zondek & Tamari (1967)	forced swimming in rats	decreased number of offspring born
Array (1967)	exposure to noise, forced swimming, shock and lights in mice	decreased fertility and increased number stillbirths
Dunlap & Vincent (1971)	exposure of cattle to to 32.2°C environment	decreased conception rate
Euker & Riegle (1973)	2 hours restraint days 1-5 in rats	decreased number implanted fetuses and number born
Thompson et al., (1982)	exposure of sheep to -2-4°C environment	decreased birthweight
Huck, Bracken & Lisk (1983)	exposure of hamsters to other pregnant female	decreased pregnancy rate and litter size
Wiebold et al., (1986)	5 hours restraint days 0-2; 3-5; 0-5 in mice	all stress regimens decreased number of implantation sites
de Catanzaro (1988)	mice housed with predator	decreased number females giving birth

measuring 13 in. x 18in. x 7in. for 6 weeks. They also discovered that under these conditions of high population density, there was a general failure of reproduction in terms of no increase in population size (Christian & LeMunyan, 1958).

The exact mechanisms of this effect could not be determined from these studies given their designs. In fact, the lack of reproductive success could have resulted from one, or a combination of, many factors which may or may not be stress related. These include: 1) disruption of sexual behaviour, 2) disturbance of the female estrous cycle and/or absence of ovulation, 3) failure of implantation of fertilized ova, or 4) spontaneous abortion or resorption of fetuses. It was thought that the inhibition of population growth was related to female factors since the males in the group did not experience reduced fertility. Males had mature, normal sperm and were capable of fertilizing the females upon discontinuation of the stress period. Despite the ambiguity, the underlying cause is thought to be stress exposure. Indeed, Calhoun (1962) suggested that there were physiological and psychological "disturbances" in the socially inhibited females in the population which caused a decrease in offspring.

Support for this idea comes from evidence suggesting that animals exposed to overcrowding show the stereotypical hormonal responses characteristic of stressors (Harvey & Chevins, 1987). Corticosterone secretion is enhanced in rats housed in large groups but not in small control grouped animals (Eechaute, Lemeester, Lacroix & Leason, 1962 cited in Christian et al., 1965; Barrett & Stockham, 1963), and increased adrenal activity is observed in overcrowded populations of

lemmings, voles and mice (Andrews, 1970). Similarly, grouped rhesus monkeys showed higher levels of 17-hydroxycorticoids than those housed alone (Mason, 1959). Physiological changes indicative of the stress response, such as enlarged adrenal glands (Christian et al., 1965) and increased blood glucose (Ader, Kreutner & Jacobs, 1963), are also seen in grouped rats but not in those housed singly.

It is known that the estrous cycles of rodents are disrupted by population stressors such as crowding, exposure to other females and in situations where many females are housed together in the absence of males or male odours (Chipman & Fox, 1966; Whitten, 1957; 1959). Overcrowding can also lead to anestrous, shortened estrous, and pseudopregnancy in different strains of mice (Dickson, 1964; Mody & Christian, 1962; Vander lee & Boot, 1955; 1956; Whitten, 1959). The estrous cycles of dairy cows can also be altered by overcrowding. For example, one study reported that shortened estrous cycles in these animals were correlated with increased herd density (MacMillan & Watson, 1971).

This stressor was also found to disrupt the pregnancies of inseminated females in Christian & LeMunyan's (1958) study. Histologic studies of the female uteri revealed implantation scars in most females despite the fact that only 3 out of 20 delivered litters. This suggests that females were fertilized by males, but later experienced intrauterine loss either in the form of imperfect implantations, or expulsion or reabsorption of fetuses later in gestation. Christian and LeMunyan (1958) suggested that the former alternative was more likely as weight gain indicative of pregnancy had not been observed in these females.

Later studies also provided support for this premise. For example, Helmreich (1960) showed that grouping caused in utero resorption of eggs in inseminated deermice.

Social Subordination

An identifiable social hierarchy exists in groups of primates. Social behaviours can be evaluated in terms of whether an animal is being aggressive, submissive or affiliative and social rank can then be assigned to individuals based on the outcomes of aggressive encounters (Sade, 1973). It has been observed that social subordination in females results in a decreased number of ovulatory cycles (Adams, Kaplan & Koritnik, 1985; Walker, Gordon & Wilson, 1983) and more cycles with disrupted ovarian hormone rhythms (Adams et al., 1985). Social subordination also leads to low levels of reproductive success for these animals in terms of number offspring born (Dittus, 1979; Sade, Cushing, Cushing, Dunaif, Figueroa, Kaplan, Laver, Rhodes & Schneider, 1976; Silk, Clark-Wheatley, Rodman & Samuels, 1981; Wilson, Gordon & Bernstein, 1978). As subordinate females were observed to have equal access to males and to engage in copulation as frequently as dominant females (Wilson, 1981), investigators have suggested that the disruption in reproductive functioning may be related to the "psychosocial stress" of being subordinate (Calhoun, 1949; Adams et al., 1985).

Similar results have been reported for rodents. As previously discussed, Calhoun (1949) housed a population of wild Norway rats in a small enclosure and observed their reproductive rate. He noted that

population size was negatively correlated with reproductive capacity and that rats of low social status showed a diminished level of reproduction. These animals conceived less often and gave birth to fewer pups than dominant females.

Handling

It has also been shown that the length of the female estrous cycle and the time of ovulation in sheep and cows can be altered by stresses such as human handling and dog herding. Furthermore, both truck transportation and exposure to new environments will have the same effect. Also, if animals' activity is restricted, there will also be a disruption of normal estrous cycling (Averill, 1964; Moberg, 1975; Williams & Wiggan, 1969).

Laboratory studies have indicated that exposure to daily handling throughout gestation, in terms of weighing and injecting with an inert substance, decreased the pregnancy rate in mice from a control value of 55% to 30% (Runner, 1959). The effect of handling on pregnancy is also thought to be stress related as handling induces biochemical responses normally associated with stressors (Kvetnansky, Sun, Lake, Thoa, Torda & Kopin, 1978).

Loud Noise

Several studies have reported that auditory stimuli induce physiological changes characteristic of the stress response. For example,

adrenal cortical weight is increased (D'Amour & Shaklee, 1955) and the stereotypical release of corticosteroids is also observed (Duncan, 1957). This is comparable to other results which indicated that auditory stimuli induced enhanced release of adrenocorticosteroids and inhibition of the gonadotropic and gonadal hormones (Sackler, Weltman & Jurtshuck, 1960; Sackler, Weltman, Bradshaw & Jurtshuck, 1959). Furthermore, intense noise has also been found to stimulate rapid ACTH release characteristic of the stress response (De Boer, van der Gugten & Slangen, 1989).

Zondek and Tamari (1960) exposed rats to an alarm bell, which rang for 1 minute at the end of each 10 minute interval, for 2 months. This treatment resulted in prolongation of the estrous cycle, as well as enhancement of ovarian size and increased numbers of corpora lutea. They also reported similar findings for rabbits (Zondek and Tamari, 1960).

These results were confirmed by Arvay (1967) who also found that intensive noise, flashing lights and intermittent shocks would cause cessation of estrous cycling, luteinization of the ovaries, and severe atrophy of reproductive anatomy including the ovaries, the follicular apparatus and neural connections to the ovaries. It should be noted, however, that such gross morphological changes are probably not the norm in response to stress as Arvay's stress regimen was severe enough to cause catatonia in the majority of exposed animals.

In what is perhaps the first controlled experiment of the effects of "stress" on pregnancy, Zondek and Tamari (1960) demonstrated that "audiogenic stimulation" could adversely affect the course of gestation.

Rats exposed to an alarm bell, which rang for 1 minute every 10 minutes for 48 hours, failed to experience implantation of fertilized embryos. This was also consistent in rabbits (Zondek and Tamari, 1967), however Stephens and Adams (1982) reported that vibration and noise stresses administered in the first week of pregnancy had no detrimental effects on gestation in their rabbits. Similarly, Hartel & Hartel (1960) found no decrease in the number of young born to rats subjected to 6 hours of intermittent bell ringing and light flashing from the 9th to the 12th day of pregnancy.

Restraint

Since Selye reported that immobilizing rats could lead to the manifestation of his physiological stress syndrome, researchers have applied a number of variations of restraint to the investigation of stress effects (see Pare & Glavin, 1986 for a review). Physical immobilization is known to produce reliable hormonal changes characteristic of stress (Barlow, et al., 1974; Barlow at al., 1975; Gibbs, 1984; Kvetnansky & Mikulaj, 1970; Riegle, 1973), and other stress-induced physiological pathologies such as weight loss (Marsh & Rasmussen, 1960) and gastric ulceration (Brodie & Hanson, 1960) without the confound of physical damage to the animal (Renaud, 1959). It is a procedure which allows the experimenter to have control over variables such as timing of stress onset, and length of the period of stress exposure, as well as avoiding infliction of tissue damage that can arise with other stressors such as scald burning and exposure to heat or cold.

One concern raised in regard to this technique is that the animals are subjected to a lengthy period of starvation which could confound the interpretation of stress effects. However, recent work has negated this as a valid concern. Barlow et al. (1975) demonstrated that corticosterone levels were not elevated in response to a 5 hour starvation period. However, corticosterone levels were elevated after restraint for 5 hours. Furthermore, Wiebold et al. (Wiebold, Stanfield, Becker, and Hillers, 1986) found no decrease in pregnancy rate, litter size, or maternal weight gain in mice deprived of food and water for 5 hours daily during the first week of pregnancy, however restraint for the same regimen did result in these effects.

With regard to the consequences of this stressor on female reproductive capacity, Euker and Reigle (1973) exposed rats to 2 hours of supine restraint, achieved by securing the animals' limbs to a countertop. This procedure was repeated daily for 3 days beginning on day 1 of diestrous. Results indicated that the expected day of estrous was delayed by 3 days implying that normal ovarian hormonal cycling was disrupted.

In pregnant rats, 2 or 3 hours of supine restraint, achieved by securing the animals' limbs to a countertop, was administered daily during different periods of gestation. This treatment resulted in significantly fewer litters born to stressed animals than to controls (Hartel & Hartel, 1960; Euker & Riegle, 1973). This result was consistent for early, middle and late pregnancy, with the greatest stress effect occurring in the latter period. Stress administered on days 1 to 5 after insemination resulted in 50% of dams giving birth, while stress on

days 5 to 12 allowed 62.5% of animals to continue to term, and day 12 to 20 application of stress resulted in 37.7% successful deliveries. Although Euker & Riegle (1973) interpreted these results to indicate that stress is more detrimental in late pregnancy, there is the confound of length of stress period. During the first period of pregnancy, animals were only subjected to five days of stress while animals stressed during the second period were exposed to eight days, and those stressed in the third experienced nine days of restraint. It is conceivable that the increased time of restraint could contribute to the prenatal loss rather than the timing of stress administration. Furthermore, based on data extrapolated from graphic representation (Euker & Riegle, 1973), there is no significant difference between stress groups. However, it is apparent that stress resulted in fewer females giving birth to litters than in the control groups during all periods of pregnancy.

Similarly, Wiebold et al. (1986), demonstrated that immobilization can block implantation and, consequently, pregnancy in mice. These investigators restrained mice for 5 hours on either the first or second three days of pregnancy, or on all six, by containing them in a wire mesh cone. Results indicated that all periods of stress reduced pregnancy rates as compared to control animals, with the six day period being more detrimental than either of the three day periods which did not differ significantly. Furthermore, morning stress was found to be more effective in blocking pregnancy than afternoon stress.

In contrast to these studies, there is one brief report that suggests that one day of immobilization stress, effected by tying the animals' limbs around a pole, was ineffective in disrupting pregnancy

when administered at different times during pregnancy (Castro-Vazquez, Esquivel, Martin & Rosner, 1975).

High Ambient Temperatures

High rates of embryonic mortality in swine (Wetteman & Bazer, 1985; Wildt, Riegle & Dukelow, 1975), sheep (Alliston & Ullberg, 1961; Alliston, Egli & Ullberg, 1961; Dutt, 1963; Yeates, 1953), dairy cattle (Ragsdale, Brody, Thompson & Worstell, 1948; Stott & Williams, 1962), and beef cattle (Dunlap & Vincent, 1971) are associated with high climatic temperatures experienced over the course of pregnancy. High ambient temperatures were found to be particularly detrimental to pregnancy in the last three months of gestation (Yeates, 1953).

Simulations of these conditions in laboratory animals have also indicated that high temperatures can inhibit ovulation and lengthen the estrous cycle (Chang & Fernandez-Cano, 1959) as well as cause embryonic loss. For example, fetal implantation in rats (Sundstroem, 1927; Fernandez-Cano, 1958; Hsu, 1948; MacFarlane et al., 1957) and mice (Pennycuik, 1966; Pennycuik, 1967) can be reduced by exposure to a 35°C environment. Furthermore, in an embryo transfer experiment, host ewes maintained in a high temperature environment experienced more losses of the normal embryos introduced than mothers living at normal temperatures (Alliston & Ulberg, 1961). A similar experiment utilizing reciprocal embryo transfer was performed in rabbits. Embryos from does housed at 96°F were transferred to recipients maintained at room temperature. Conversely, the embryos of the normal temperature

group were introduced to the heated females. Results indicated that fetal viability was decreased in both groups, however thirty-five per cent of the heated embryos survived in normal mothers while less than 10 per cent of the normal embryos lived in the heated does. Consequently, Shah (1956) suggested that the effects of heat stress were mediated through maternal physiology rather than directly on the embryo.

Similarly, extremely low temperature environments such as at 4°C or less can also lead to disruption of pregnancy in rats (Fernandez-Cano, 1958) and sheep (Thompson, Bassett, Samson & Slee, 1982).

Although it has been suggested that pregnancy is disrupted at abnormal temperatures due to alterations in the rate at which different biological processes occur (Hsu, 1948), it is generally accepted that the effect is stress mediated. It is known that changes in surrounding temperature will cause a pattern of corticosterone release (Christison, 1972) and ACTH (Marple, Jones, Alliston & Forrest, 1974) characteristic of stress. Furthermore, laboratory studies on sheep exposed to high temperatures demonstrated that the increased light and alterations in food supply associated with high temperature seasons were not responsible for the failure of pregnancy (MacFarlane, Pennycuik, Yeates and Thrift, 1959).

In contrast, there is one study suggesting that the stress caused by high or low ambient temperatures will not cause fetal loss in rats (Castro-Vazquez, Esquivel, Martin & Rosner, 1975). These investigators found no effects on the pregnancy of rats subjected to 7 hours of extreme heat or cold on one day at various times during pregnancy.

Exposure to Novel Males

Exposure of recently inseminated mice to novel males will cause a disruption of pregnancy in laboratory mice (Bruce, 1959; 1960), wild mice (Eleftheriou, Bronson & Zarrow, 1962; Heske & Nelson, 1984), and voles (Storey, 1986). This result, known as the Bruce Effect, may not be stress-related, however. It has been suggested that the olfactory pattern produced by the first male discriminates him from other males and the olfactory stimulus released by a new male will be recognized as unfamiliar by the female consequently inducing a pregnancy block (Bruce, 1963; Parkes & Bruce, 1962). This pheromonal hypothesis is supported by demonstrations that merely exposing the females to cages soiled by novel males (Parkes & Bruce, 1962), or to their urine (Dominic, 1965; 1966a; 1966b) is sufficient to produce a pregnancy block. Furthermore, if females are made anosmic by surgical transection of the olfactory tract, exposure to a novel male will not result in pregnancy failure (Rajendren & Dominic, 1986).

One interesting twist on the Bruce effect involved exposing recently inseminated hamsters to other pregnant females. Results indicated that the subordinate female in the pair gave birth to significantly fewer offspring, if any, than the other female. This effect, however, was only consistent for pairs who had engaged in aggressive activity suggesting that there may be a behavioural component involved (Huck, Bracken & Lisk, 1983).

Other evidence also supports the role of behavioural interactions in the mediation of the Bruce effect. deCatanzaro and Storey (1989) suggested that mating behaviour on the part of the new male may affect the reproductive endocrinology of the female, thus disrupting pregnancy. Their experiments supported this proposal by showing that the amount of sexual activity exhibited by the new male was negatively correlated with the number of litters born (deCatanzaro & Storey, 1989). Thus, it is possible that the Bruce effect is mediated by both pheromonal and behavioural factors related to the novel male, and that the behaviour may be stressful to the female.

Exposure to a Predator

Other experiments examined the effects of housing recently inseminated mice with rats which are natural predators of mice. A substantial proportion of rats will exhibit a stereotyped killing response toward mice (Bandler and Moyer, 1970; Karli, 1956), and thus mice might be expected to experience psychological stress when confronted with rats. This naturalistic form of stress resulted in a decrease in the number of females giving birth to live litters and in the total number of pups born in CD-1 and C57 mice (deCatanzaro, 1989; MacNiven & deCatanzaro, 1990), but not in heterogeneous strain (HS) mice (MacNiven & deCatanzaro, 1990).

Exposure to Novel Environments

One report indicates that pregnant ewes transferred to a novel environment, or exposed to herding by a dog within the first twenty days after mating show greater pregnancy failure rates than those ewes left undisturbed (Doney, Smith & Gunn, 1976).

Similarly, recently inseminated deermice which are exposed to changes in their social environment, will produce fewer litters than those not exposed. Such conditions include the presence of other mice versus isolation, or alterations in physical surroundings in terms of changing the mice to a novel cage and/or changing cage size (Bronson, Eleftheriou & Garick, 1964; Eleftheriou et al., 1962).

Other Stressors

A number of other miscellaneous stimuli have been used to induce physiological stress responses in pregnant animals. For example daily sessions of forced swimming will block early pregnancy in rats (Zondek and Tamari, 1967). Similarly, complex stressors which combine a number of stimuli are also detrimental to pregnancy. Arvay (1967) exposed animals to an alarm and bright lights for ten minutes, followed by ten minutes of forced swimming, and finally ten minutes of unpredictable footshock. Predictably, this treatment lead to fewer females giving birth to litters and to an increased number of stillbirths.

Another complex paradigm utilizing forced swimming followed by exposure to heat, bright light and random tones also resulted in a disruption of pregnancy in mice (Weir and DeFries, 1963).

Summary and Conclusions

Apart from two studies reviewed (Castro-Vazquez et al., 1975; Hartel & Hartel, 1960), it appears that the evidence is overwhelmingly supportive of the idea that stress administered in early pregnancy will result in fetal loss in mammals. Furthermore, the effect of stressors on any period of pregnancy is "all-or-none". This means that there is a decrease in the per cent of females giving birth under stressful conditions in comparison to unstressed dams. There is no reduction in litter size or weight, or weight per pup for each dam as a result of stressor exposure (Euker & Reigle, 1973; Wiebold et al., 1986; MacNiven & deCatanzaro, 1990).

CHAPTER THREE

Physiological Mechanisms Underlying Stress-Induced Pregnancy Blocks

Involvement of Hypothalamic-pituitary-adrenal Mechanisms

Given that the hypothalamic-pituitary-adrenal hormones are important to both the stress response and pregnancy viability, it is logical that they have been implicated in the etiology of stress-induced failure of reproduction. A number of studies which involve the manipulation of the stress-related physiological systems suggest that ACTH or glucocorticoids can have adverse effects on several stages of the normal reproductive process.

ACTH has been given much attention as it is an important regulator in the actions of the pituitary-adrenocortical axis, and directly controls corticosterone output. One study reported that administration of physiological levels of ACTH can disrupt menstrual cycles in baboons (Rowell, 1970). Comparable results have been demonstrated in rodents as ACTH was found to disturb the normal estrous cycling in rats (Hagino, Watanabe & Goldzieher, 1969) and immature mice (Christian, 1971), as well as to block ovulation in rats (Hagino et al., 1969). Such effects have also been found with ACTH, as well as cortisone acetate, in sheep (Doney, Gunn, Smith & Carr, 1976).

Similarly, ACTH injections administered 5 days before mating, as well as on day 7 and on days 11-15 of pregnancy will result in an increased incidence of stillbirths and decreased litter size in rats

on days 1 to 3 of pregnancy in that a decreased number of embryos will implant. It is interesting to note, however, that the time interval required for the ova to reach the uterus was unaffected (Yang, Yang & Lin, 1965). In mice, ACTH administered during either the first or second half of pregnancy, or throughout gestation also caused spontaneous abortions, fetal resorption, and reduced fetal growth and litter size in both intact and adrenalectomized mice (Kittinger, Gutierrez-Cernosek, Cernosek & Pasley, 1980). This effect has also been reported in rabbits (Robson and Sharaf, 1952).

Glucocorticoids have also been implicated in the prevention of implantation (Bitman, and Cecil, 1967; Schlough, 1971; Velardo, Hisaw & Bever, 1956). Injections of cortisone acetate increased the fetal resorption rate in rats. Furthermore, cortisone administered during heat stress resulted in a potentiation of the fetal loss effect seen with heat alone (MacFarlane et al., 1957).

Evidence also indicates that other adrenal steroids, androstenedione (A4) and dehydroepiandosterone (DHEA), which are both released from the adrenal during stress (Fenske, 1986; Fuller, Hobson, Reyes, Winter & Faiman, 1984), can cause an expulsion of fertilized eggs from the reproductive tract and diminish the number of pups delivered after insemination (Harper, 1967, 1969). As this effect is thought to be related to the conversion of these steroids to estrogen, the results will be discussed later within the context of estrogen effects on pregnancy.

Despite reports on the detrimental effects of ACTH and cortisone acetate on pregnancy, there is reason to question the role of

hypothalamic-pituitary-adrenal hormones in blocking early pregnancy. Sandman and coworkers (Sandman, Kastin, Achally, Kendall & Miller, 1973) reported that ACTH was increased in male rats in response to inescapable footshock, but not in a conditioned emotional response paradigm. Similarly, Mason (1974) demonstrated that the neuroendocrine profile obtained in rhesus monkeys in response to different noxious stimuli varied dramatically. These results suggest that ACTH response to stress may vary depending on the nature of the stressor. Furthermore, no effects of ACTH on pregnancy were noted in one experiment utilizing adrenalectomized rats suggesting that adrenal mechanisms may be involved, but any effects of ACTH are probably secondary (Velardo, 1957).

Although Barlow et al. (1975) demonstrated that a single exposure to surgical stress during pregnancy increased corticosterone levels substantially, Euker & Riegle (1973) reported no deleterious effects of repeated surgeries on pregnancy outcome. Furthermore, previous studies in this laboratory have demonstrated that exogenous corticosterone is without effect on early pregnancy in mice (deCatanzaro, MacNiven & Ricciuti, 1990).

As in the case of adrenalectomy abolishing stress induced ulcers, it is reasonable to presume that if stress induced pregnancy loss is related to adrenal hormones, then adrenalectomy should reverse pregnancy blocks due to stress. Of two studies examining the effect of adrenalectomy upon strange male induced pregnancy disruption, one (Snyder & Taggert, 1967) reported that this manipulation diminished the block, while the other (Sahu & Dominic, 1981) reported a failure of this

surgery in preventing the block. Furthermore, Hensleigh & Johnson (1971a; 1971b) found that removal of the adrenal gland in stressed pregnant animals did not counteract the adverse effects of stress on pregnancy, but that removal of the ovaries followed by replacement estrogen and progesterone administration abolished the effects of heat stress on fetal development. These authors suggested that the stressor may have direct effects on pituitary-gonadal functioning which resulted in pregnancy disruption and that the effects of stress-induced adrenal secretions were minimal. Thus, it may well be due to an alteration in the ratio of progesterone to estrogen that pregnancy can fail in response to environmental or psychological stress (Gidley-Baird, O'Neill, Sinosich, Porter, Pike, and Saunders, 1986).

Indeed, there are some good reasons why this may be the case. First, the effect on pregnancy obtained through administration of ACTH is not comparable to that induced by the application of a stressor in that it is not an "all-or-none" effect. As previously stated, stressors result in a decrease in the number of females giving birth rather than in a reduction in litter size for each dam (Euker & Reigle, 1973; Wiebold et al., 1986; MacNiven & deCatanzaro, 1990). However, the effects of estrogen administration on early pregnancy are consistent with those of stress (Edgren & Shipley, 1961; Chang & Yanagamachi, 1965; Deanesley, 1963; deCatanzaro, et al., 1990; Greenwald, 1961; 1963; Huet & Dey, 1987; Pincus & Kirsch, 1936; Stone & Emmens, 1964). This, coupled with the lack of effect of corticosterone as well as the inconclusive effects of adrenalectomy, suggests that adrenal steroids may be less important

than gonadal hormones in the induction of stress-induced pregnancy loss.

Hormonal Control in Normal Pregnancy

Although the physiological mechanisms through which psychological stress impedes early pregnancy have not yet been established, the hormones directly responsible for embryonic viability during this period are known. Prior to implantation, the mammalian embryo is free to travel through the fallopian tubes and is surrounded by oviductal or uterine fluids containing the maternal hormones necessary for implantation to occur (Cowan, Manes & Hagerman, 1976; Borland, Erickson & Ducibella, 1977; Fowler, Johnson, Walters & Eager, 1977). The major steroid hormones involved in the establishment and maintenance of pregnancy are 17B-estradiol and progesterone which are released from the maternal ovaries (Gidley-Baird, 1981), as well as the adrenocorticoids and aldosterone (Grota and Eik-nes, 1967).

Additionally, a specific ratio of estrogen to progesterone is essential for the development of embryos and their subsequent transportation through the fallopian tubes (Smith & Biggers, 1968). These two hormones act synergistically to control the rate of embryonic travel and the precise time of implantation (Roblero and Garavagno, 1979). The normal pattern of progesterone secretion in rats and mice involves the following sequence. Estrogen levels tend to be very low during the implantation stage with the exception of a short period late on day 4 when a surge occurs. Estrogen levels then return to the former

low levels until they begin to climb towards the end of the middle third of pregnancy, to reach a high plateau close to parturition (Gidley-Baird, 1981; McCormack & Greenwald, 1974; Nalbandov 1971; Shelesynak & Kraicer, 1962; Yoshinaga, Hawkins & Stocker, 1969). Progesterone and estrogen from alternate sources, such as the fertilized egg, have also been discounted as important for the maintenance of pregnancy due to the extremely small amounts contributed (Dickmann, Dey, and Gupta, 1976).

Progesterone Involvement

Progesterone is instrumental in allowing implantation to occur and is also necessary for the maintenance of pregnancy after implantation (Deanesley, 1966; McCormack and Greenwald, 1974).

Many investigators have reported that pregnancy will fail without adequate progesterone levels, due to a variety of progesterone depleting manipulations. Bilateral ovariectomy performed at any point during pregnancy will lead to abortion in many animal species. Progesterone administration preceding and following the surgery at any stage in gestation can forestall fetal loss in rats (Csapo and Resch, 1979; Csapo and Wiest, 1969), guinea pigs (Deanesley, 1972), musk shrews (Hasler and Nalbandov, 1978), mice (Hall, 1956; 1957; Humphrey, 1967; McLaren, 1971; Rubinstein and Forbes, 1963), and hamsters (Orsini and Meyer, 1962). It should be noted that many precursors and metabolites of progesterone have been found to be ineffective in preventing pregnancy

loss after ovariectomy (Forbes, 1967; Weist & Forbes, 1964) suggesting great response specificity.

As nutrition in early pregnancy and progesterone levels are inversely correlated in ewes (Parr, Cumming & Clarke, 1982), it is logical that overfeeding may lead to lowered progesterone levels and consequent abortion. Working from this premise, Parr and coworkers (Parr, Davis, Fairclough & Miles, 1987) demonstrated that exogenous progesterone administration could counteract detrimental effects of overfeeding, allowing more pregnancies to continue to term.

Other investigators have found that transfer of fertilized rabbit eggs from a normal donor into a recipient pretreated with long acting progestins will result in an increased pregnancy rate as compared to untreated controls (Chang & Saksena, 1983).

In mice genetically selected for their inability to produce litters of normal size, it is apparent that ovulation rate is normal, and the observed decrease in litter size is due to prenatal mortality. One study showed that serum progesterone concentrations during pregnancy in these animals are lower than in a strain producing normal litters. Furthermore, exogenous progesterone given on days 6 to 12 of gestation can also enhance litter size in the selected strain (Michael, Geschwind, Bradford, and Stabenfeldt, 1975).

Pregnancy will also be interrupted if progesterone levels are decreased by administration of a monoclonal antibody to progesterone. This has been shown in mice (Rider, McRae, Heap, & Feinstein, 1985; Wang, Rider, Heap & Feinstein, 1984), and ferrets (Rider & Heap, 1986).

Effects of Stressors on Progesterone

There are few reports examining the effects of stress on endogenous progesterone levels, however existing evidence suggests that stress decreases progesterone levels in pregnant baboons (Albrecht, Nightengale, and Townsley, 1978). In baboons subjected to the stress of restraint and tranquilization for 90 minutes on one morning in late pregnancy, plasma progesterone levels decreased over the stress period. Unfortunately, there is no information on the effects of stress on progesterone in early pregnancy in this species, and it is not known what pattern progesterone secretion took after this ninety minute period. This is important as another investigator found that heat stress caused a decrease in progesterone levels in swine when applied in late pregnancy, however an enhancement of progesterone was apparent in response to early gestational heat stress (Wettemann & Bazer, 1985).

Diminished progesterone levels have also been reported after the application of restraint stress for the first 5 days of early pregnancy in mice (Wiebold et al, 1986). Nevertheless, only half of the stressed animals actually exhibited diminished progesterone levels. Also, although progesterone level appeared to be associated with success of pregnancy, half of the animals experiencing progesterone depletion still had intact implanted embryos. Consequently, it is difficult to discern whether decreases in progesterone are directly responsible for the stress-induced pregnancy losses observed in this experiment.

Other investigators have reported that the biochemical consequences of stressful stimuli can elevate progesterone levels. It is

known that ACTH administration can increase plasma progesterone (Piva, Gagliano, Motta and Martini, 1977; Resko, 1969) and administration of dexamethasone can counteract this effect (Resko, 1969). In ovariectomized rats and guinea pigs, ACTH administration causes increased plasma progesterone levels sufficient to facilitate female sexual receptivity (Feder & Ruf, 1969).

It has also been shown that the application of stressors can enhance progesterone secretion. For example, a two hour restraint period was found to increase serum progesterone levels in nonpregnant rhesus monkeys (Fuller et al., 1984). The same restraint regimen also produced serum progesterone elevations in rats (Miller and Riegle, 1985), and a 6 hour confinement period resulted in increased progesterone secretion in gerbils (Fenske, 1986).

The application of acute stress in the form of a 5, 15 or 90 minute exposure period to a -10°C environment, or ether anesthesia, also increased adrenal output of both pregnenolone and progesterone in rats (Holzbauer & Newport, 1967). Similar results were found under the influence of a mild chronic stress induced by replacing the normal drinking water of female rats with 0.9% saline for 1 month. This group of animals secreted four times more progesterone, from both the adrenal and ovaries, and one and a half times more corticosterone than unstressed controls. This increase in progesterone output was also true for male and female rats exposed to sham surgery, with the chronically stressed group secreting more progesterone than the acute animals (Fajer, Holzbauer & Newport, 1971).

There is also evidence indicating that stress increases progesterone in pregnant animals. Dunlap and Vincent (1971) reported a direct relationship between increased rectal temperature of heifers under heat stress and elevated progesterone levels. This relationship was also positively correlated with decreased pregancy rate.

Stress induced by irregular signalled shock for 10 minutes resulted in significantly higher progesterone levels in rats exposed to one session and in rats exposed each day throughout pregancy (Pollard and Dyer, 1981). Thus, it is apparent that the response of progesterone to stress is unclear.

Furthermore, there are few demonstrations that exogenous progesterone can sustain pregnancy in animals exposed to stressful stimuli. There is one brief abstract (Runner, 1959) indicating that the previously described disruption of pregnancy in mice by human handling can be prevented by exogenous progesterone. Although this report presents no quantitative details, Runner (1959) does suggest 2 mg of progesterone injected daily will not only offset the deleterious effects of handling, but will enhance pregnancy rate to above that of the control group. It should be noted that the control pregnancy rate was only 55% and progesterone administration in stressed animals boosted their pregnancy rate from 30% to 75%.

Administration of progesterone to rats housed in a 35°C environment also resulted in a small reduction of fetal loss, but heated animals still experienced more than three times as many reabsorptions as controls (MacFarlane et al., 1957)

It has also been reported that exogenous progesterone will maintain pregnancy that otherwise would be disrupted by exposure to the urine of novel males (Dominic, 1966a; 1966b), however, as previously stated, there is no reason to believe that pregnancy loss incurred in this manner is stress-induced. Furthermore, reinstatement of pregnancy with progesterone is limited to a specific time frame beyond which progesterone has no effect (Rajendren and Dominic, 1987).

Estrogen Involvement

Given that estrogen is present in very small quantities for the majority of the early gestational period, it is not surprising that excessive estrogen levels are known to be detrimental to pregnancy. It has been demonstrated that several different forms of natural and synthetic estrogen administered during the first third of pregnancy will disrupt implantation. This outcome was first demonstrated in the rat and mouse by Parkes and Bellerby (1926) and Smith (1926) respectively. It is interesting to note that although estrogen has powerful deleterious effects on early pregnancy, it is apparently ineffective in causing interruption of gestation after implantation has occurred (Levin, Katzman & Doisy, 1931; D'Amour & Shacklee, 1955). Thus, it is apparent that estrogen must act on the preimplantational physiology of either the embryo, the mother, or both.

The physiological mediators of this effect have been extensively investigated. Estrogenic substances were found to have adverse effects on the migration of fertilized ova through the fallopian tubes in that large

amounts caused acceleration of ova transport, while smaller doses lead to retention of ova in the fallopian tubes which later degenerated (Burdick & Whitney, 1937; Burdick, Whitney,& Pincus, 1937; Burdick, Emerson & Whitney, 1940; Burdick & Veder, 1941; Whitney and Burdick, 1936; Chang and Yangamachi, 1965; Greenwald, 1965)

This effect has been confirmed in a number of species. As well as in a number of strains of rats (Banik & Pincus, 1965; Dreisbach, 1959), estrogens have also been found to interrupt early pregnancy in Badgers (Edgren & Shipley, 1961), rabbits (Chang & Yanagamachi, 1965; Greenwald, 1961; 1963; Pincus & Kirsch, 1936), guinea pigs (Deanesley, 1963), hamsters (Chang & Yanagamachi, 1965), and mice (Stone & Emmens, 1964; Huet & Dey, 1987; deCatanzaro, MacNiven & Ricciuti, 1990). Furthermore, Noyes and coworkers (Noyes, Adams, & Walton, 1959) found that transfer of fertilized rabbit eggs from a normal donor into an estrogen treated host resulted in rapid expulsion of the eggs suggesting that estrogen acts on the maternal physiology rather than directly on the corpus lutea.

Further evidence that high maternal estrogen levels are related to fetal loss comes from the agricultural literature. In a study investigating the etiology of pregnancy loss in cattle, it was observed that whereas there was no difference in progesterone levels between pregnant cows as compared to those who failed to produce viable embryos, estrogen levels were higher 3 to 4 days after mating in those who lost conceptuses (Ayalon, 1973).

In contrast to all of the studies which report an effect of estrogen on implantation, one study reported no effect of a single dose of estrogen administered in early pregnancy (Black & Asdell, 1959).

Converging evidence for the adverse effects of estrogen on early pregnancy comes from studies examining implantation after in vitro fertilization. Results indicate that high estrogen levels are associated with embryonic loss in both mice (Gidley-Baird et al., 1986) and humans (Lewinthal, Mahadevan, Pattinson, Taylor & Persaud, 1987; Gidley-Baird, et al., 1986).

Other research suggests that the adrenal steroids androstenedione (AD) and dehydroepiandosterone (DHEA), which are released during stressful stimuli (Fenske, 1986; Fuller et al., 1984), can also have detrimental effects on the preimplantation stage of pregnancy. Harper (1967) reported that subcutaneous administration of 4 or 8 mg of AD per day on days 1 to 4 of pregnancy could interfere with implantation in rats. Further investigation demonstrated that implantation was prevented because the eggs had been quickly expelled from the reproductive tract. Thus, it was not a uterine change that resulted in the loss of pregnancy. This response is very similar to that induced by the administration of estrogens (Whitney & Burdick, 1936). This is not surprising as AD is known to be metabolized to estrogen (Dorfman & Sharma, 1965) and in fact Harper suggested that it is the conversion to estrogen that lends AD its pregnancy blocking effects.

Similarly, DHEA, a precursor to AD, can also block implantation in rats when administered in doses of 2.5, 5, and 10 mg daily on days 1 to 4 of pregnancy. The mechanism in this case was also

found to be accelerated migration and subsequent expulsion of the eggs prior to implantation. Thus, DHEA is probably detrimental to pregnancy also due to its conversion to estrogen in the ovary (Harper, 1969). As both of these hormones are known to be released during stress (Fenske, 1986; Fuller et al., 1984), it is conceivable that they are then converted to estrogens which interfere with egg implantation resulting in a stress-induced pregnancy block.

Effects of Stressors on Estrogen

There is a growing body of evidence which suggests that administration of ACTH can result in increased endogenous estrogen levels in nonpregnant animals (Arai, Kuwabara and Okinaga, 1972) and in late pregnancy in humans (Strott, Sundel, & Stahlman, 1975). Simmer and coworkers (1974) found that ACTH causes endogenous estrogen to increase over the first 4 to 7 hours after injection in humans in late pregnancy (Simmer, Tulchinsky, Gold, Frankland, Greipel & Gold, 1974). Thus, it is conceivable that the rise in ACTH incurred during stressor exposure may also result in enhanced estrogen levels. Furthermore, as AD and DHEA are released during a stressful experience. and given that these steroids are readily synthesized to estrogen, it is possible that estrogen levels may increase under stress due to the biochemical converion of AD and DHEA.

There are currently no published studies examining the possible effects of stress on endogenous estrogen levels during pregnancy. One experimenter suggested that heat stress in pregnant

swine may lead to increased estrogen levels during one period of pregnancy and decreased levels as compared to unheated controls at another thus giving inconclusive results (Wettemann & Bazer, 1985).

Scattered reports in the human literature suggest that increased estrogen levels are correlated with anxiety in nonpregnant women (Backstrom & Mattson, 1975), especially in those reporting premenstrual syndrome symptoms (Backstrom & Carstensen, 1974; Backstom, Sodergaard & Carstensen, 1976). Furthermore, there is one paper which reports that women with polycystic ovary syndrome may experience higher stress levels than normal women, and that these women also have higher endogenous levels of estrogen than normals (Lobo, Granger, Paul, Goebelsmann, and Mishell, 1983). Although these studies are purely correlational in nature, it is interesting to speculate that the observed increases in estrogen levels may be related to stress.

Statement of Hypotheses

As previously stated, there is reason to challenge the existing belief that stress-induced pregnancy blocks are mediated through adrenal hormone secretions in response to the stressor. Recall that corticosterone has no deleterious effects on pregnancy whatsoever, and that ACTH does not produce the "all-or-none" effect on litter size that is seen in response to a stressor. Also, adrenalectomy does not appear to prevent stress-induced pregnancy loss. Conversely, the effects of estrogen administration are consistent with the effects of stress on early pregnancy (Edgren & Shipley, 1961; Chang & Yanagamachi, 1965; Deanesley, 1963; deCatanzaro, et al., 1990; Greenwald, 1961; 1963; Huet & Dey, 1987; Pincus & Kirsch, 1936; Stone & Emmens, 1964). Furthermore, it is possible that stressed-induced pregnancy blocks may be reversed through exogenous progesterone administration (MacFarlane et al., 1957; Runner, 1959). These inconsistancies suggest that adrenal steroids may be less important than gonadal hormones in the induction of stress-induced pregnancy loss. The purpose of this thesis was to investigate the effects of stress on estrogen and progesterone and the roles of these hormones in the disruption of pregnancy under stressful circumstances.

Hypothesis 1:

The fact that progesterone is essential for implantation and maintenance of early pregnancy implies that any interference with the synthesis or action of progesterone in early gestation will lead to pregnancy loss. Furthermore, according to Selye's (Selye & Heuser, 1955) account of the stress response, the pituitary should shift from producing gonadotrophic hormones to ACTH and adrenal steroids when a stressor is introduced. This would result in decreased progesterone output from the ovary. Thus, the first hypothesis investigated was that the application of an external stressor leads to diminished progesterone levels which causes a failure of implantation of fertilized eggs and consequent pregnancy loss.

Hypothesis 2:

Since estrogen has such a dramatic adverse effect on early pregnancy at such a low exogenous dose, it is conceivable that only a small enhancement of the endogenous level by psychological stressors could interrupt pregnancy. Since there is evidence that ACTH stimulation increases estrogen (Arai et al, 1972) and that the adrenal androgens released during stress (Fenske, 1986; Fuller et al., 1984) are converted to estrogen (Harper, 1967), there are at least two mechanisms by which this could occur. Consequently, the second hypothesis was that demanding stimuli result in an increase of endogenous maternal

estrogen levels which could account for the negative influences of such stressors on gestation.

Note: At the commencement of the experimental work for this thesis, reliable methods for the radioimmunoassay of gonadal steroids were not readily available. Consequently, the initial experiments described herein are indirect approximations of the hypotheses, while the concluding project did involve direct hormone analysis.

CHAPTER FOUR

PROGESTERONE EXPERIMENTS

Experiment 1

Introduction

In order to the examine the hormonal mechanisms underlying stress-induced pregnancy block, it was necessary to establish a reliable, controllable stress paradigm. As previously discussed, physical immobilization is known to produce the hormonal changes characteristic of stress (Barlow, et al., 1974; Barlow at al., 1975; Gibbs, 1984; Kvetnansky & Mikulaj, 1970; Riegle, 1973) and other stress-induced physiological pathologies (Brodie & Hanson, 1960; Marsh & Rasmussen, 1960). This procedure allows the experimenter to have control over many aspects of unavoidable stress exposure including timing of stress onset and length of exposure time. Furthermore, there is a large body of literature on the use of this technique for other applications which can be consulted in order to aid in determining the ideal parameters. Experiment 1 investigated whether stress induced by supine restraint administered during the first week after insemination would block pregnancy in Heterogeneous Strain (HS) mice.

Subjects

Subjects were female HS mice bred in our own colony from stock originally obtained from the Department of Zoology at the University of Toronto. After weaning and prior to insemination, females were housed in groups of 4 or 5 in standard polypropylene cages measuring 28 x 16 x 11 (height) cm with wire grid tops allowing continuous access to food and water. The colony room was maintained under a reversed 10:14 hr dark/light cycle and at 21 $\pm$ 1°C. Isolated HS males were also housed under these conditions.

Methods

Insemination Procedure

When the females were at least 65 days of age, they were each housed alone at the commencement of the dark phase of the light cycle with one male of the same strain in a cage like that described above. The males were sexually experienced and had been deprived of access to a female for at least 7 days. After every 2-1/2 hour period following pairing, the hindquarters of each female were examined for the presence of a sperm plug. The day of detection was designated as day 0 of pregnancy. At the commencement of the next dark phase, the female was removed, weighed, and assigned to one of the experimental or control conditions. Assignment to conditions was counterbalanced across date of insemination.

Stress Procedure

Sixteen female mice were subjected to 5 hours of restraint on each of days 1 to 5 after insemination. The animals were immobilized using a method developed by Renaud (1959). Each was fixed in a prone position to a specially constructed restraint board using surgical tape. Animals appeared physically undamaged, provided with water, and maintained in a dark room in correspondence with their light cycle. The restraint treatment was administered during the period 1-6 hours after commencement of the dark phase of their lighting cycle. A 5 hour period was chosen because of previous findings that the maximum hormonal effect of this stimulus is reached at 1 hour after commencement and is maintained for the following 6 hours (Barlow et al., 1975), and that 5 hours of restraint at the beginning of the dark cycle is the optimal regimen for stress effects in pregnancy (Wiebold et al., 1986). After each restraint session the animals were reinstated to their respective cages. Following the last session, the animals were again returned to their cages and then were not disturbed until day 18 when they were weighed and checks for litters began. Sixteen control subjects were inseminated and placed in clean cages in isolation; they were not disturbed at all until day 18.

Outcome Measures

In both conditions, females were housed alone throughout the course of the experiment. On the eighteenth day after insemination, they

were weighed again and thereafter visually inspected on at least four occasions each day for the occurrence of birth. Date of birth, number of pups born, number of stillbirths, number of pups surviving to three days of age, and total surviving litter weight three days after birth were recorded for each female. A blunt wooden prod was used to gently move pups for counting without leaving human odors. Stillborn or partially cannibalized pups were removed by hand using a surgical glove. All of these measures were taken in order to be as comprehensive as possible, however the measure of number of pups surviving to day three was redundant with the number born as there were no significant differences between these measures in any of the experiments.

Results

Subjective Observation of Organismic Responses to Stress Procedure

All of the animals survived in apparent good health during the restraint period. During the course of the restraint procedure, the animals tended to lie quietly if undisturbed. If a light was turned on in order to check their well-being, many animals responded with vocalizations and wriggled around in the restraint device. Many animals also chewed at the padding covering their limbs or on the restraint board itself. Occasionally, an animal would succeed in releasing a limb, which was replaced upon discovery.

When released at the end of the stress period. animals engaged almost immediately in grooming behaviours, usually followed by eating and drinking. All animals appeared to behave normally, in terms of

activity level, when replaced in their home cages. No abnormal maternal behaviours were noted in stressed mothers who delivered litters. There was also no significant difference between groups in the length of term.

Results

Tables 3 and 4 show all measures of pregnancy outcome and litter vitality. There were fewer dams giving birth in the stressed condition than in the control group. There were also fewer litters among the restrained animals than among controls. An analysis of variance was performed to investigate whether there was a significant difference between conditions in terms of the selected outcome measures for all subjects. A value of 0.0 for the number of pups born, and a litter weight of 0g were assigned to females who had failed to deliver a litter. There were significant effects of condition for number of pups born, $F(1,27)=11.46$, $p=.0025$; the number surviving to day 3 after birth, $F(1,27),=9.87$, $p=.0043$; and for litter weight at day 3, $F(1,27)=13.56$, $p=.0013$. A Chi square test of association between the presence or absence of pregnancy and experimental conditions was conducted. This analysis showed a significant rejection of the hypothesis that pregnancy outcome was unrelated to condition, $X^2(1)=13.60$, $p<.001$.

Table 3

Sample Sizes, Percent of Females Bearing Litters, Percent of Pups Cannibalized, and Percent of Pups Stillborn for Each Condition in Experiment 1

Group	n	% of females with litters	% pups cann.	% stillborn
restraint	13	38	0	0
control	16	100	1	3

Table 4

Means (± S.E.) of Measures of Maternal Weight (g) at Day 18, Number of Pups Born, Number Surviving to Day 3, Litter Weight at Day 3 (g) and Weight Per Pup (g) for Experiment 1

Group	Mat.Wt. Day 18	# Pups Born	# Pups Day 3	Litter Wt. Day 3	X Wt.(g) Per Pup
restraint	31.9±3.2[a]	4.0±1.5	4.0±1.5	9.6±3.5	2.4±0.2
control	47.1±2.0[a]	8.7±0.8	8.4±0.8	22.0±1.9	2.7±0.0

[a]Based on less than the full n for each condition due to parturition on day 18 of pregnancy.

Experiment 2

Introduction

Experiment 1 demonstrated that the application of chronic restraint can interrupt early pregnancy in HS mice. This is consistent with the aforementioned reports which indicate that a variety of natural and laboratory "stressors " are detrimental to reproductive outcome. Consequently, the application of chronic stress in the form of inescapable restraint was chosen as a paradigm for studying the physiological events underlying stress-induced pregnancy loss. Some previously cited reports have suggested that stress experienced during early pregnancy may result in diminished levels of progesterone (Albrecht et al., 1978; Wiebold et al., 1986) which is essential for pregnancy maintenance (Roblero & Garavagno, 1979). It is therefore possible that diminished progesterone levels contribute to stress-induced pregnancy interruptions.

If such pregnancy blocks are caused by inadequate progesterone levels induced by stress, then replacement progesterone should reverse the effect and allow pregnancy to continue to term. The purpose of this study was to examine the pregnancy blocking effects of restraint on two different strains of mice, and whether a daily dose of progesterone would counteract the adverse effects of restraint stress on pregnancy outcome.

Subjects

Subjects were 30 female HS mice, and 44 C57 female mice obtained from Canadian Breeding Farms, LaPrairie, Quebec. Subjects were housed. prepared and inseminated as described for Experiment 1.

Methods

Females were mated with males of their own strain. At the commencement of the next dark phase of the light cycle, subjects were weighed and randomly assigned to one of four experimental conditions.

In the first condition, commencing on day 1 of pregnancy, animals were injected subcutaneously with 500 ug progesterone dissolved in .05 cc peanut oil and then immediately placed in the restraint device as previously described. This procedure was repeated daily up to and including day 5 of pregnancy. Animals in the second condition received the same treatment, except that the injections consisted only of the oil vehicle. In the third condition, subjects were only placed in the restraint devices daily. The fourth condition was simply a control group in which, after being weighed, the animals were placed singly in clean cages. All outcome measures were also as outlined in Experiment 1.

Results

Figure 1 shows the mean number of pups born in each condition. Tables 5 and 6 provide all other measures of maternal weight at day 18, number of pups born, number of pups at day three after birth, and surviving litter weight at day 3. Analyses of variance were performed on maternal weight at day 18, number of pups born, number surviving to day 3, and the litter weight at day 3. Again, a value of 0.0 for the number of pups born, and a litter weight of 0g were assigned to females who had failed to deliver a litter. For all four measures, there were significant effects of condition [maternal weight, $F(3,106)=6.42$, $p=.0008$; number born, $F(3,106)=3.83$, $p=.0120$; number at day 3, $F(3,106)=3.76$, $p=.0130$; and litter weight, $F(3,106)=4.15$, $p=.0081$]. The effect of strain was also significant for each of these measures [maternal weight, $F(1,106)=19.20$, $p=.0001$; number born, $F(1,106)=13.74$, $p=.0006$; number at day 3, $F(1,106)=13.89$, $p=.0006$; and litter weight, $F(1,106)=19.86$, $p=.0001$]. Restraint was clearly effective in reducing the number of live births in both strains. Although the C57 females were apparently less responsive to exogenous progesterone than were the HS females, none of the interactions in these analyses reached significance. Duncan's multiple comparisons ($p<.05$) were conducted for all of these measures. For maternal weight, the HS control and progesterone treated groups on the one hand significantly differed from the HS vehicle and restraint groups on the other hand. For both number born and weight on day 3, the HS control group differed from the HS restraint and oil groups, while the HS progesterone group differed from the HS

Table 5

Sample Sizes, Percent of Females Bearing Litters, Percent of Pups Cannibalized, and Percent of Pups Stillborn for all Conditions in Experiment 2

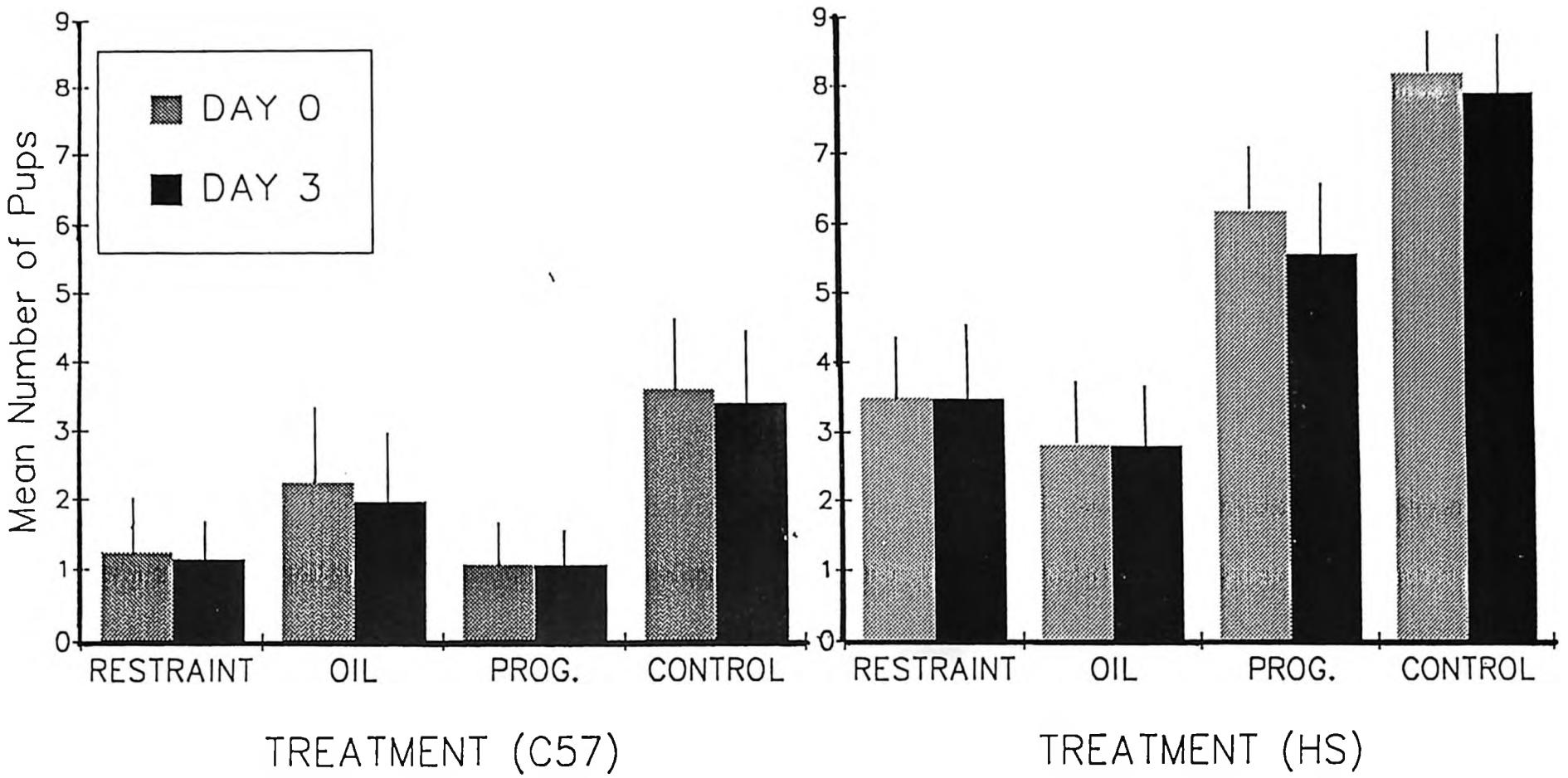
Group	n	% of females with litters	% pups cann.	% stillborn
C57				
restraint	10	20	0	0
restraint + oil	11	18	0	12
restraint + prog.	11	27	0	7
control	11	55	5	0
HS				
restraint	18	33	0	0
restraint + oil	18	28	0	0
restraint + prog.	17	65	6	4
control	18	83	3	1

Table 6

Means (± S.E.) of Measures of Maternal Weight on Day 18, Number of Pups Born, Number Surviving to Day 3, Mean Litter Weight on Day 3, and Mean Weight Per Pup for all Conditions in Experiment 2

Group	Mat. Wt. Day 18	# Pups Born	# Pups Day 3	Lit. Wt. Day 3	$\bar{X}$ Wt. Per Pup
C57					
restraint	25.8±1.9	1.3±0.9	1.3±0.9	2.2±1.5	1.9±0.3
restraint + oil	28.2±2.3	2.3±1.2	2.0±1.1	3.9±2.2	1.9±0.2
restraint + prog.	26.2±1.6	1.1±0.7	1.1±0.7	1.9±1.3	1.7±0.0
control	33.0±2.7	3.6±1.1	3.5±1.0	7.3±2.2	2.1±0.0
HS					
restraint	31.0±2.7	3.5±1.2	3.5±1.2	8.5±3.0	2.4±0.2
restraint + oil	30.9±2.5	2.8±1.1	2.8±1.1	6.9±2.8	2.4±0.0
restraint + prog.	39.0±2.4	6.2±1.2	5.6±1.1	15.1±3.1	2.8±0.1
control	44.0±2.3	8.2±1.0	8.2±1.0	20.7±2.7	2.6±0.0

Figure 1: The mean (±SE) number of pups born and surviving to day 3 produced by C57 and HS female mice after exposure to physical restraint and treatment with progesterone or vehicle in Experiment 2.


Mean Number of Pups
DAY 0
DAY 3
0
1
2
3
4
5
6
7
8
9
RESTRAINT
OIL
PROG.
CONTROL
TREATMENT (C57)
RESTRAINT
OIL
PROG.
CONTROL
TREATMENT (HS)

oil group. For the number surviving to day 3, the HS control group differed from the restraint and oil groups. For none of these measures were there differences among the C57 groups, although most C57 groups differed from the comparable HS groups. A Chi square test of association between the presence or absence of pregnancy and experimental conditions showed a significant rejection of the hypothesis that pregnancy outcome was unrelated to condition $X^2(7)=24.09, p<.005$.

Experiment 3

Introduction

Experiment 1 showed that chronic restraint can block pregnancy. Experiment 2 demonstrated that the deleterious effects of restraint stress on pregnancy can be partially counteracted by the administration of exogenous progesterone in one strain of mice. In order to test the generality of the effects of stress on pregnancy and the role of progesterone, another type of stressor was examined. The stress used was predator exposure. As discussed previously, a large proportion of rats will exhibit a stereotyped killing response when exposed to a mouse (Bandler and Moyer, 1970; Karli, 1956). In a previous study on C57 mice in this laboratory, 84.6% of the control animals produced litters while only 9.1% of the rat-exposed group delivered successfully (deCatanzaro, 1989).

The purpose of this experiment was to examine whether administration of increasing concentrations of progesterone could reverse the pregnancy block induced in mice by rat exposure.

Subjects

C57 female mice were obtained from Canadian Breeding Farms, LaPrairie, Quebec, and were housed, prepared and inseminated as previously described in Experiment 1. HS mice were not examined in

this experiment because a preliminary experiment indicated that their pregnancy is not vulnerable to rat exposure.

Methods

Females mice were inseminated as in Experiment 1. At the commencement of the next dark phase of the light cycle, subjects were weighed and randomly assigned to one of five experimental conditions. The first condition consisted of no-treatment controls who, following assignment to this group, were simply placed individually in standard cages and left alone until the pregnancy outcome measures were taken. All of the remaining conditions involved exposure of the inseminated females to preselected nonassaultive rats continuously for 6 days commencing on day 1 after detection of the sperm plug.

Stress Procedure

The rat exposure cages were standard double hanging rat cages partitioned in half by wire mesh with squares of 0.625 cm sides. A rat, previously selected as nonassaultive was placed on one side of the cage. The partition allowed independent and free access to food and water for both the rat and the mouse. A full description of the procedure can be found in deCatanzaro. (1989). The first of these rat-exposure conditions involved no other treatment. The second rat-exposed group received a daily s.c. injection of .05 cc oil at 3 hours after commencement of the dark phase of the lighting cycle. The third rat-exposed group received

such injections containing 100 ug of progesterone, while in the fourth, the injections contained 500 ug progesterone. On day 7 after insemination, each rat exposed female was transferred to a clean mouse cage as described above and left undisturbed until pregnancy outcome measures. Such measures were as in Experiment 1, except that maternal weight at day 18 was not recorded.

Results

Figure 2 shows the number of pups born per inseminated female. Tables 7 and 8 report all remaining measures of pregnancy outcome. Rat exposure blocked pregnancy completely in both the stress and oil groups. Progesterone appeared to have a positive effect in counteracting the pregnancy block, especially at the higher dosage. Analysis of variance was conducted on each of three measures: number of pups born, number of live pups on day 3 after birth, and the litter weight on day 3. Again, a value of 0.0 for the number of pups born, and a litter weight of 0g were assigned to females who had failed to deliver a litter.

There was a significant effect of treatment apparent in each of these measures [number born, $F(4,52)=7.72$, $p=.0002$; number at day 3, $F(4,52)=7.80$, $p=.0002$; and litter weight at day 3, $F(4,52)=7.57$, $p=.0002$]. Multiple comparisons indicated that for all measures, the oil vehicle and the rat exposure alone groups on the one hand differed from the 500 ug progesterone and no treatment control on the other, and that the 100 ug progesterone group differed from the no treatment control. A Chi square

Table 7

Sample Sizes, Percent of Females Bearing Litters, Percent of Pups Cannibalized, and Percent of Pups Stillborn for all Conditions in Experiment 3

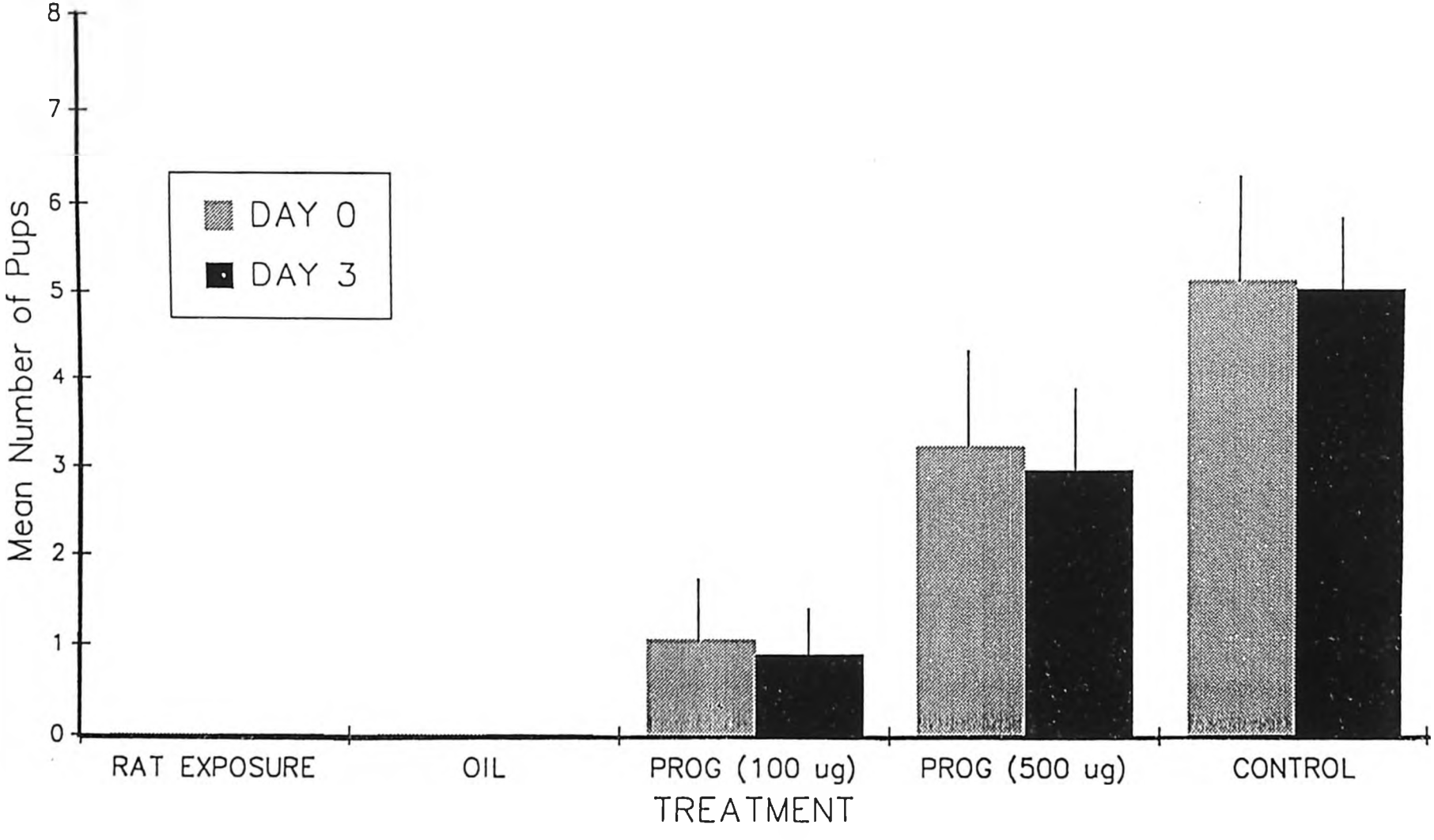
Group	n	% of females with litters	% pups cann.	% stillborn
rat exposure	12	0	0	0
rat exposure + oil	11	0	0	0
rat exposure + 100ug prog.	12	25	14	0
rat exposure + 500ug prog.	11	45	0	8
control	10	70	2	0

Table 8

Means (± S.E.) of Number of Pups Born, Number Surviving to Day 3, Mean Litter Weight (g) on Day 3, and Mean Weight Per Pup (g) for all Conditions in Experiment 3

Group	# Pups Born	# Pups Day 3	Lit. Wt. Day 3	$\bar{X}$ Wt. Per Pup
rat exposure	0	0	0	___
rat exposure + oil	0	0	0	___
rat exposure + 100ug prog.	4.7±2.3	4.0+2.3	8.9+5.1	1.5+0.7
rat exposure + 500ug prog.	7.2+0.6	6.6+1.0	14.7+2.5	2.2+0.0
control	7.4+0.8	7.3+0.8	15.9+1.9	2.2+0.0

Figure 2: The mean (±SE) number of pups born and surviving to day 3 produced by C57 female mice after exposure to a rat and treatment with progesterone or vehicle in Experiment 3.


Mean Number of Pups
8
7
6
5
4
3
2
1
0
DAY 0
DAY 3
RAT EXPOSURE
OIL
PROG (100 ug)
PROG (500 ug)
CONTROL
TREATMENT

test of association between pregnant and nonpregnant versus experimental condition showed a significant rejection of the hypothesis that pregnancy outcome was unrelated to condition, $X^2(7)=24.09, p<.005$.

Discussion

This experiment replicates previous findings from this laboratory that predator exposure will lead to termination of pregnancy in C57 mice (deCatanzaro, 1989). This work is extended in this experiment as it is now apparent that progesterone is partially effective in helping to sustain pregnancy blocked by the stress of exposure to a predator. This is also consistent with the findings of previous investigators (Dominic, 1966; Hall, 1956; 1957).

Experiment 4

Introduction

Given that the results to this point suggest that progesterone levels in early pregnancy may be decreased in response to stress, a possible mechanism underlying this reaction was investigated. It is conceivable that stress exerts its effects by acting on any of a number of levels of the hypothalamic-pituitary-gonadal axis, however another possibility exists. Corticosterone, the primary hormone released in rodents during stress, is synthesized from progesterone. As the rat adrenal gland only contains the amount of corticosterone secreted in the first 2-3 minutes of stressor application, there must be an increase in corticosterone production during prolonged stressor exposure (Holzbauer & Newport, 1967). Furthermore, the adrenal gland also releases large amounts of pregnenolone and progesterone under stressful circumstances (Holzbauer & Newport, 1967), and evidence suggests that ACTH has a stimulatory effect on the transformation of cholesterol to pregnenolone (Dorfman & Unger, 1965; Stone & Hechter, 1954). Consequently, it is important to determine if progesterone is synthesized to sustain the corticosterone release induced by stress, resulting in lowered progesterone levels and consequent fetal loss. *2-methyl-1,2-bis(3-pyridyl)-1-propanone*, also known as metyrapone, is a potent inhibitor of this biochemical process (Dominiquez & Samuels, 1963), and as indicated from evidence regarding sexual receptivity, its administration appears to preserve natural progesterone levels

(deCatanzaro, Knipping, and Wigmore, 1983; deCatanzaro, 1985). Consequently, an experiment was performed to examine whether a daily dose of metyrapone would counteract the adverse effects of restraint stress on pregnancy outcome.

Subjects

Subjects were 75 female HS mice, bred in our laboratory. Prior to insemination, females were housed in groups of 4 or 5. When the females were at least 65 days of age, they were inseminated as described for previous experiments.

Methods

Five groups of subjects were prepared. The groups consisted of an untreated control group, a vehicle injection control group, and three doses of metyrapone [Ciba] 400, 500, and 600 ug per subcutaneous injection, each dissolved in .05 cc propylene glycol. All injections were administered daily immediately before placement in the restraint device about one hour after commencement of the dark phase of the lighting cycle. The injections and restraint regimen began on day one of pregnancy and continued through day five. From day 6 after insemination onward, all animals were left undisturbed until pregnancy outcome measures were taken, which included maternal weight at day 18, number of pups born, number surviving to day 3, the litter weight at day 3, and the mean weight per pup.

Results

Tables 9 and 10 present the measures of pregnancy outcome. Restraint was effective in blocking pregnancy, and metyrapone was effective in reinstating it. Analyses of variance were performed and revealed significant effects of condition on maternal weight at day 18, $F(4,70)=4.16$, $p=0.005$, number of pups born, $F(4,70)=3.59$, $p=0.01$, number surviving to day 3, $F(4,70)=3.90$, $p=0.007$, and the litter weight at day 3, $F(4,70)=4.77$, $p=0.002$. Again, a value of 0.0 for the number of pups born, and a litter weight of 0g were assigned to females who had failed to deliver a litter. Duncan's multiple comparisons ($p < .05$) were conducted for all outcome measures. For maternal weight at day 18, the control group on the one hand significantly differed from the restraint and treatment groups on the other hand. None of the other measures showed significant differences. A Chi square test of association between the preseence or absence of pregnancy and experimental conditions showed a significant rejection of the hypothesis that pregnancy outcome was unrelated to condition, $X^2(4)=13.41, p<.01$.

Discussion

Akin to the effects of progesterone shown in Experiment 3, metyrapone also partially reinstated pregnancy in stressed mice, although this effect was not as strong as that of progesterone. It is therefore possible that chronic stress may not exert its effects on

Table 9

Sample Sizes, Percent of Females Bearing Litters, Percent of Pups Cannibalized, and Percent of Pups Stillborn for all Conditions in Experiment 4

Group	n	% of females with litters	% pups Cann.	% stillborn
restraint + vehicle	15	33	2	0
restraint + 0.4ug mety.	15	60	0	0
restraint + 0.5ug mety.	15	60	0	0
restraint + 0.6ug mety.	15	40	8	0
control	15	93	2	0

Table 10

Means (+ S.E.) of Measures of Maternal Weight on Day 18 (g) , Number of Pups Born, Number Surviving to Day 3, Mean Litter Weight on Day 3 (g), and Mean Weight Per Pup (g) for all Conditions in Experiment 4

group	Mat. Wt. Day 18	# Pups Born	# Pups Day 3	Lit.Wt. Day 3	$\bar{X}$ Wt. Per Pup
restraint + vehicle	47.4±2.3	9.8±0.9	9.2±0.6	17.5±2.0	1.9±0.1
restraint + 0.4ug mety.	47.3±1.9	10.3±0.6	9.3±1.3	19.0±1.1	1.8±0.0
restraint + 0.5ug mety.	49.2±1.3	9.8±0.5	9.6±0.5	18.4±0.9	1.9±0.0
restraint + 0.6ug mety.	50.8±1.5	10.5±0.4	9.7±0.3	17.2±0.8	1.8±0.0
control	50.2±1.0	10.1±0.5	9.9±0.4	19.6±0.7	2.0±0.0

progesterone levels by inhibiting some branch of the hypothalamic-pituitary-gonadal axis. Instead, under conditions of chronic stress, progesterone may be rapidly converted biochemically in order to maintain corticosterone output. Indeed, it is known that the rat adrenal gland only stores enough corticosterone to sustain output for the first 2-3 minutes of organismic challenge (Holzbauer & Newport, 1967). Consequently, it is possible that adrenal production of corticosterone is maintained at the expense of progesterone. This may offer one explanation for the purpose of enhanced adrenal progesterone secretion under stressful circumstances.

It should be noted that the highest dose of metyrapone failed to reinstate pregnancy despite the success of the lower doses. This suggests that there may be a ceiling effect for the potency of metyrapone. This is supported by previous findings that metyrapone has a very limited dose range within which it exerts stimulatory effects on sexual behaviour (deCatanzaro et al., 1983; deCatanzaro, 1985).

Summary and Discussion of Progesterone Experiments

Experiments 1 through 4 demonstrate clearly that both physical restraint stress and rat exposure block early pregnancy in C57 mice, however only restraint is effective in the HS strain. The effect of physical restraint upon early pregnancy demonstrated in Experiments 1, 2 and 4 is consistent with past reports (Euker and Riegle, 1973; Wiebold et al., 1986), and the effect of predator exposure shown in Experiment 3 supports previous findings from this laboratory (deCatanzaro, 1989). In each of these cases, there was no reduction of litter size in stressed animals who carried to term. Rather, there was an "all-or-none" effect in that fewer stressed animals delivered litters relative to controls. Furthermore, for all of these experiments, there were no differences in the measures of litter vitality such as the number of pups stillborn or cannibalized, and the mean weight of pups surviving to day three.

Furthermore, Experiments 2 through 4 show that bolstering endogenous progesterone levels through the concomitant administration of progesterone or metyrapone will partially protect pregnancy from the harmful effects of the stressors, with progesterone being somewhat more effective. Hall (1956; 1957) reported a similar observation in experiments of handling in late pregnancy. Dominic (1966) also cited the effectiveness of progesterone in reinstating pregnancy, but the study was a small adjunct focussing on other issues. Additionally, Csapo and Wiest (1969) examined the quantitative relationship between pregnancy maintenance with progesterone following ovariectomy, but to my

knowledge, there have been no systematic reports of this kind in mice, especially regarding stress manipulations in early pregnancy.

One anomalous outcome seen is that exogenous progesterone was effective for the partial inhibition of the restraint induced pregnancy block in HS, but not in C57 subjects. The reasons for this strain difference are unclear, especially since progesterone was effective in counteracting the effects of rat exposure. Wiebold et al. (1986) reported that mice which were stressed and continued to term were significantly heavier on day 0 than those losing their litters. Since it is known that body fat is correlated with ovulation rate (Fowler and Edwards, 1960), Wiebold et al. (1986) proposed that the heavier mice completed pregnancy despite stress, due to higher numbers of ovulations and resultant corpora lutea which provided a protection mechanism. Since the corpora lutea secrete progesterone, these mice may have had higher endogenous progesterone levels which aided in counteracting the negative effects of stress. In this study, the C57 group were significantly lighter on the average than the HS group. Thus, perhaps higher exogenous progesterone doses should be given to C57 subjects in order to account for their presumably lower endogenous levels. Support for this proposal emerges from an analysis of variance which shows an interaction between day 0 maternal body weight and pregnancy outcome in each condition.

The results of Experiment 2 through 4 could be interpreted to support the suggestion that stress reduces progesterone levels in early pregnancy (Albrecht et al., 1978; Wiebold et al., 1986). Although there

are reports to the contrary (Fenske, 1986; Fajer et al., 1971; Fuller et al., 1984; Holzbauer & Newport, 1967; Miller and Riegle, 1985; Pollard and Dyer, 1981; Vincent, 1971), it may be that both of these opposing views are viable. It is possible that the pattern of progesterone secretion resulting from stress may be variable over time and thus the time at which blood sampling occurs after the onset of the stressor may have a bearing on the progesterone level. Indeed, under normal physiological conditions there is a diurnal rhythm to the progesterone levels of pregnant rodents upon which the changes induced by pregnancy are imposed (Thompson et al., 1975).

CHAPTER FIVE

ESTROGEN EXPERIMENTS

Introduction

Only partial pregnancy reinstatement is seen when progesterone levels are preserved through exogenous administration of progesterone or metyrapone. This suggests that if progesterone depletion is a mechanism underlying the pregnancy block effect, it must not be the sole mechanism. Other hormonal factors must play a role in the maintenance and disruption of early pregnancy.

Estrogen is an extremely effective pregnancy blocking agent (Whitney and Burdick, 1936; Chang and Yangamachi, 1965; Greenwald, 1965; deCatanzaro et al., 1991). Furthermore, there is evidence to suggest that ACTH results in estrogen release (Arai et al, 1972; Simmer et al, 1983; Strott et al., 1975). Consequently, it is possible that stress may cause an enhancement of endogenous estrogen which may lead to pregnancy loss. The possible role of estrogen in the stress-induced pregnancy block effect was investigated in the second series of experiments.

Experiment 5

Introduction

Following replication in C57 and HS mice of the finding that estrogen is a potent blocker of pregnancy, it was thought that it would be valuable to determine the minimum effective dose of this compound, as well as to plot a dose response curve.

Subjects and Methods

Female HS mice were prepared and inseminated following procedures described for Experiment 1. The only exception to this was that upon detection of a sperm plug, females were immediately removed, weighed, and assigned to groups. Nine groups of subjects were prepared. The groups consisted of an untreated control group, a vehicle injection control group, and seven dosages of 17β-estradiol: 0.004, 0.013, 0.037, 0.111, 0.333, 1.000, and 3.000 υg per injection, each dissolved in .05 cc peanut oil. All injections were administered daily at about 5 hours after commencement of the dark phase of the lighting cycle. The injections began on day one of pregnancy and continued through day six.

Upon completion of the treatment regimen, the animals were left undisturbed until the eighteenth day following insemination. At this time, they were weighed, as previously described, and thereafter inspected on at least two occasions each day for the occurrence of birth.

The remaining outcome measures were also identical to those specified in Experiment 1.

Results

Figure 3 gives the results for number of pups born. Table 11 gives the number of pups per litter, the number of pups surviving to day 3, and the total litter weight on day 3 after birth. Table 12 gives the percentages of females giving birth in each condition, as well as the number of pups stillborn and cannibalized. It is clear that pregnancy was completely blocked at daily dosages of 0.333 ug and greater. There were a few completed pregnancies at a daily dosage of 0.111 ug. Most females produced apparently normal litters at the 0.037 ug dosage, while females receiving lower dosage still were indistinguishable from controls. The data were tabulated and a value of 0.0 for the number of pups born, and a litter weight of 0g were assigned to females who had failed to deliver a litter. Analyses of variance showed significant effects of condition for number of pups born $F(8,79)=22.5$, $p < .0001$, number of pups alive on day 3, $F(8,79)=22.77$, $p < .0001$. On each of these measures, Newman-Keuls multiple comparisons showed that in all cases, dosages of 0.111 ug and greater differed significantly from all dosages below that point. A Chi square test of association between pregnant and nonpregnant versus experimental condition showed a significant rejection of the hypothesis that pregnancy outcome was unrelated to condition, $X^2(8)=34.14$, $p<.001$. It should be noted that

Table 11

Means (±S.E.) of Measures of Number of Pups Born (Num. Born) Number of Pups Surviving to Day 3 after Birth (Num. 3), and Litter Weight at Day 3 (Lit. Wt.), after Exposure to Varied Dosages of Unmodified Estradiol-17b in Experiment 5

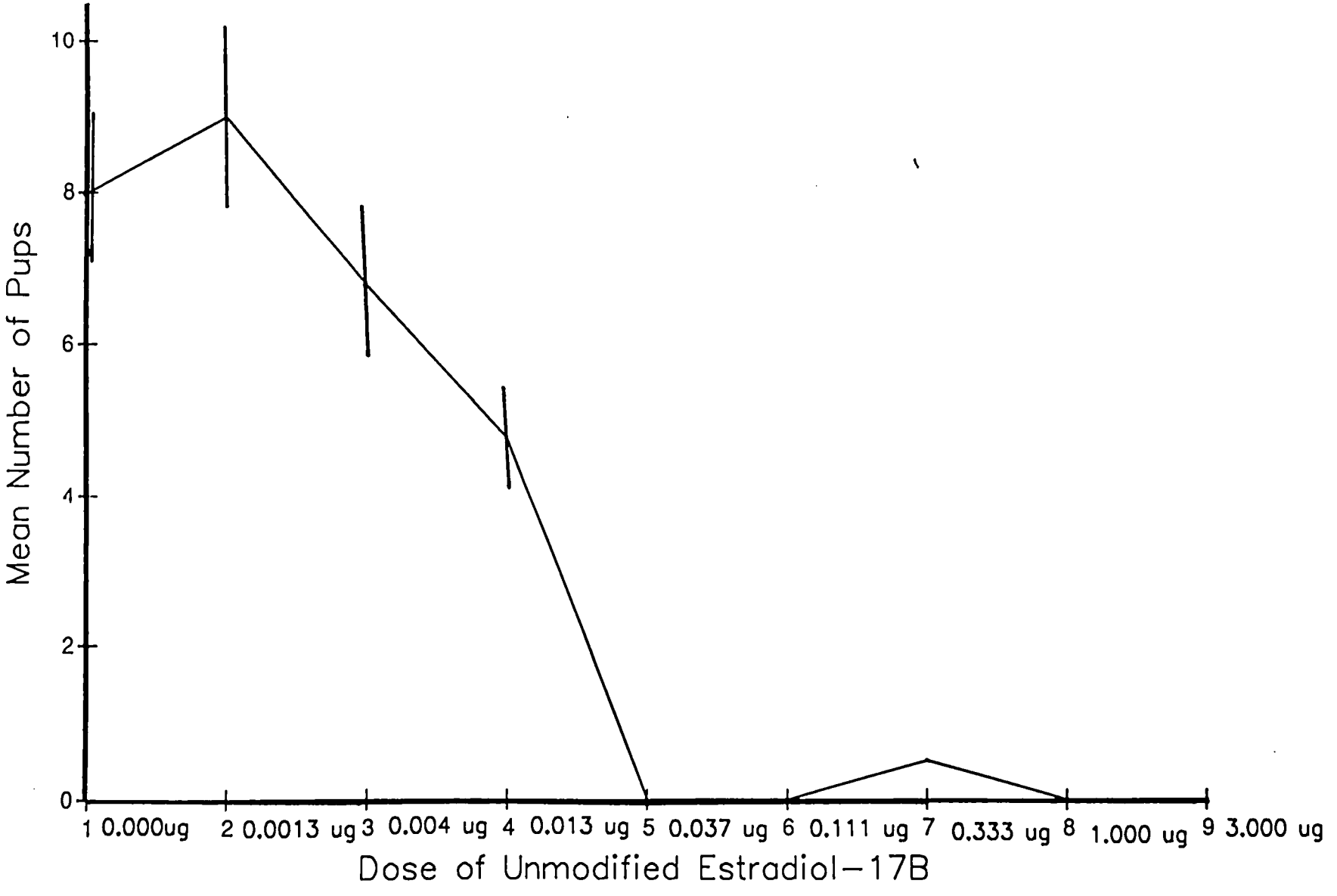
Dose	Num. Born	Num. 3	Lit. Wt.	n
0.000 ug	8.0±1.6	7.9±1.6	16.7±3.2	7
0.0013 ug	9.0±1.9	9.0±1.9	17.6±3.5	6
0.004 ug	6.8±2.2	6.8±2.2	16.3±5.4	6
0.013 ug	4.8±2.0	4.8±2.0	12.4±5.1	5
0.037 ug	0.0±0.0	0.0±0.0	0.0±0.0	6
0.111 ug	0.0±0.0	0.0±0.0	0.0±0.0	6
0.333 ug	0.5±0.5	0.5±0.5	1.7±1.7	6
1.000 ug	0.0±0.0	0.0±0.0	0.0±0.0	7
3.000 ug	0.0±0.0	0.0±0.0	0.0±0.0	7

Table 12

Sample Sizes, Percent of Females Bearing Litters, Percent of Pups Cannibalized, and Percent of Pups Stillborn After Exposure to Varied Dosages of Unmodified Estradiol-17b in Experiment 5

Dose	n	% of Females with Litters	% of Pups Cann.	% of Pups Stillborn
0.000ug	7	86	2	0
0.0013ug	6	83	0	0
0.004ug	6	67	0	0
0.013ug	5	60	0	0
0.037ug	6	0	0	0
0.111ug	6	0	0	0
0.333ug	6	17	0	0
1.000ug	7	0	0	0
3.000ug	7	0	0	0

Figure 3: The mean (±S.E.) number of pups born after administration of varied daily doses of unmodified estradiol-17β in the first period of pregnancy in Experiment 5.


Mean Number of Pups
10
8
6
4
2
0
1 0.000ug
2 0.0013 ug
3 0.004 ug
4 0.013 ug
5 0.037 ug
6 0.111 ug
7 0.333 ug
8 1.000 ug
9 3.000 ug
Dose of Unmodified Estradiol-17B

a timing study was also done to determine the period of early pregnancy most sensitive to estrogen. Results indicated that an intermediate dose of estrogen (0.111 ug) administered for two consecutive days during any of the first six days of gestation resulted in a complete block of pregnancy.

Experiment 6

Introduction

As shown in Experiment 5, it is clear that 17β-estradiol is extremely potent in blocking early pregnancy. This natural estrogen is the end product of the biosynthethesis of both dehydroepiandosterone and androstenedione, the adrenal androgens released under stressful circumstances (Fenske, 1986). This synthesis pathway may account for the reported pregnancy blocking properties of these androgens (Harper 1969a; 1969b; deCatanzaro et al., 1991). Nevertheless, previous studies of the pregnancy blocking effects of estrogen have focussed on synthetic estrogens (Edgren & Shipley, 1961; Chang & Yanagamachi, 1965; Greenwald, 1961; 1963; Huet & Dey, 1987; Pincus & Kirsch, 1936; Stone & Emmens, 1964). Furthermore, there are no reported dose response data for a synthetic estrogen in early pregnancy. Consequently, for comparison, a second dose response curve was done using the synthetic estrogen, estradiol-17β benzoate.

Subjects and Methods

Female HS mice were inseminated following procedures described previously. On detection of a sperm plug, females were immediately removed, weighed, and assigned to groups. Nine groups of subjects were prepared. The groups consisted of an untreated control, a vehicle injection control group, and seven doses of estradiol-17β benzoate: 0.004, 0.013, 0.037, 0.111, 0.333, 1.0, and 3.0 υg per injection. Each dose was dissolved in 0.05 cc peanut oil. All injections were administered daily at about 5 hours after commencement of the dark phase of the lighting cycle. The injections began on day one of pregnancy and continued through day 6. The outcome measures taken were consistent with those specified in Experiment 5.

Results

Figure 4 gives the results for number of pups born. Table 13 shows the maternal weight on day 18, the mean number of pups surviving to day 3, and the total litter weight on day 3 after birth. A value of 0.0 for the number of pups born, and a litter weight of 0g were assigned to females who had failed to deliver a litter. Table 14 gives the percentages of females giving birth, and the mean number of pups stillborn, and cannibalized in each condition. It is clear that pregnancy was completely blocked at daily dosages of 0.333 υg and greater, and there were only two completed pregnancies at a daily dosages of 0.111 υg. Most females produced normal litters at the 0.037 υg dose, while females receiving

Table 13

:ans (±S.E.) of Measures of Maternal Weight at Day 18 of Pregnancy (Mat. Wt.), Number of Pups Surviving to Day 3 after Birth (Num. 3), and Mean Litter Weight at Day 3 after Birth (Lit. Wt.) after Exposure to Varied Dosages of Estradiol-17b Benzoate in Experiment 6

:c	Mat. Wt.	Num. 3	Lit. Wt.
ıtrol	48.0±9.6[a]	9.9±1.6	23.5±3.6
)0 ug	42.6±13.2	6.8±1.5	16.5±3.6
)4 ug	46.1±10.3[a]	9.1±1.3	22.1±3.7
13 ug	49.2±6.6	10.9±0.4	23.9±1.1
37 ug	40.9±9.7	7.1±1.6	16.4±3.7
11 ug	29.3±5.1[a]	0.4±0.3	1.1±0.9
33 ug	27.1±1.6	0.0±0.0	0.0±0.0
)0 ug	27.0±2.0	0.0±0.0	0.0±0.0
)0 ug	26.5±0.8	0.0±0.0	0.0±0.0

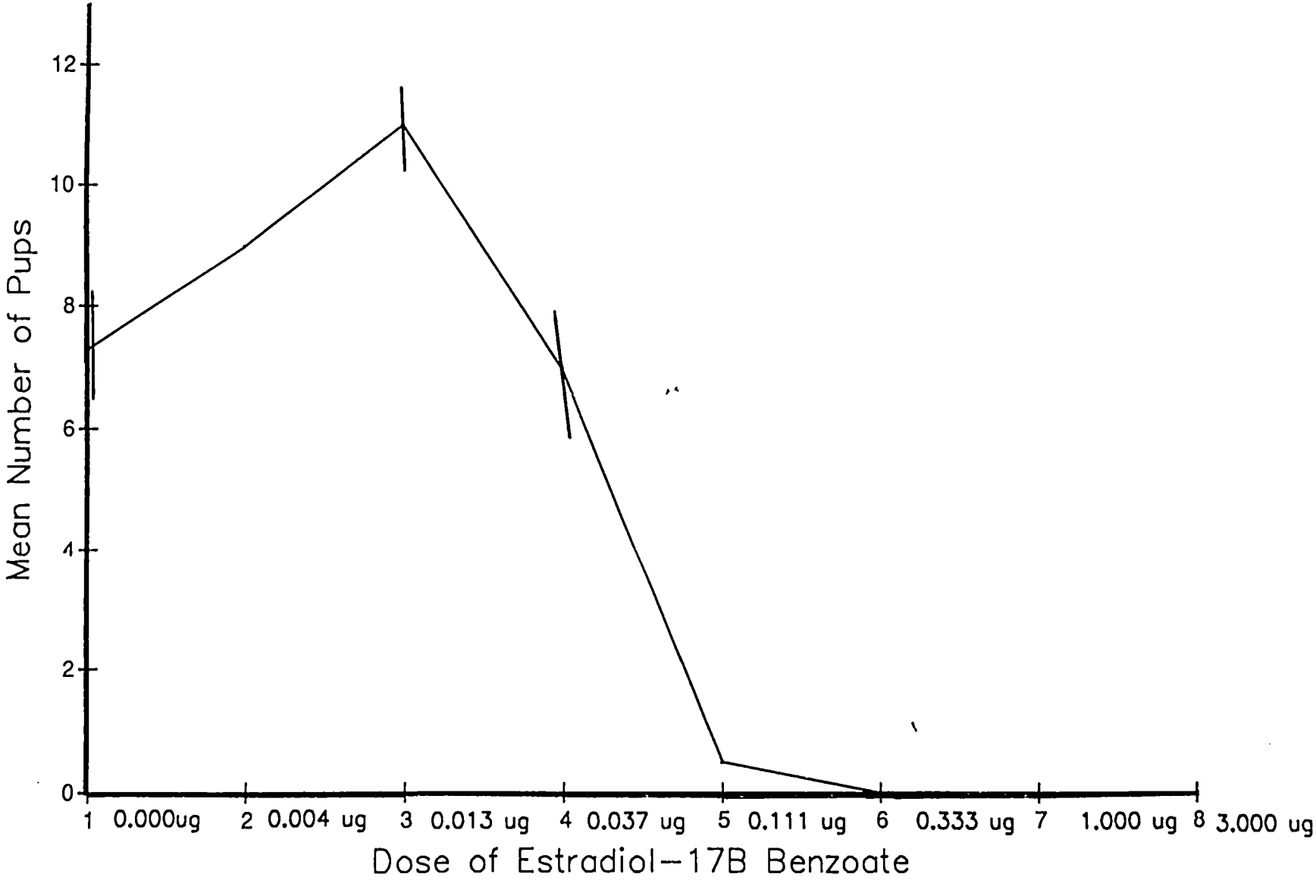
sed on one dam less than the full condition n due to parturition on day 18 of pregnancy.

Table 14

Sample Sizes, Percent of Females Bearing Litters (% Litters), Percent of Pups Cannibalized (% Cannib.), and Percent of Pups Stillborn (% Still.) after Exposure to Varied Dosages of Estradiol-17b Benzoate in Experiment 6

Dose	n	% Litters	% Cannib.	% Still.
Control	8	87	2	2
0.000 ug	10	70	0	7
0.004 ug	10	90	0	2
0.013 ug	10	100	0	2
0.037 ug	10	70	0	0
0.111 ug	10	20	17	17
0.333 ug	10	0	-	-
1.000 ug	10	0	-	-
3.000 ug	10	0	-	-

Figure 4: The mean (±S.E.) number of pups born after administration of varied daily doses of estradiol-17β benzoate in the first period of pregnancy in Experiment 6.


Mean Number of Pups
12
10
8
6
4
2
0
1 0.000ug
2 0.004 ug
3 0.013 ug
4 0.037 ug
5 0.111 ug
6 0.333 ug
7 1.000 ug
8 3.000 ug
Dose of Estradiol-17B Benzoate

lower doses were indistinguishable from controls. Analyses of variance showed significant effects of condition for number of pups born, $F(8,79)=22.50$, $p<.0001$; number of pups alive on day 3, $F(8,70)=22.57$, $p<.0001$; and total litter weight on day 3, $F(8,79)=22.77$, $p<.0001$. On each of these measures, Newman-Keuls multiple comparisons showed that doses of 0.111 µg and greater on the one hand differed significantly from all doses below that point on the other hand. None of the conditions within these sets differed significantly, except that the 0.013 µg condition showed significantly greater numbers of pups born and surviving to day 3 compared to the oil vehicle group. A Chi square test of association was significant $X^2(8)=57.64$, $p<.001$, suggesting a rejection of the hypothesis that pregnancy outcome was unrelated to condition.

Discussion

These dose response data are consistent with similar, although less detailed, results found previously. Greenwald (1965) compared the state of the corpora lutea in hamsters treated with 1 µg, 25 µg, 50 µg, 100 µg, and 250 µg of estradiol cyclopentylpropionate. Despite limitations such as small sample size, the results indicated that the first two doses had no apparent effect, while the others resulted in the regression of the corpora lutea in all subjects. Other studies have reported comparable findings (Burdick and Whitney, 1937; Chang and Yanagamachi, 1965; Whitney and Burdick, 1936), although not in this strain of mice.

It is clear that estrogen acts in a predictable dose-response relationship. That is, at high doses, there are no litters, while at

intermediate doses, there appears to be a partial pregnancy block, with no block being seen at extremely low doses. These "high" doses, however, are still extremely small; in fact, it was found that estrogen blocked pregnancy at a dose which was one thousandth of the lowest dose of dehydroepiandrosterone used and one five-thousandth of the minimal effective dose of androstenedione (deCatanzaro et al., 1990). In view of the sensitivity of pregnancy to even small amounts of estrogen, it is conceivable that only a small enhancement of endogenous levels by stress could account for the pregnancy block effect seen under stressful circumstances.

Experiment 7

If pregnancy block is caused by excessive estrogen levels induced by stress, then decreasing estrogen levels through the administration of an agent that reduces circulating estrogen levels should reverse the effect and allow pregnancy to continue to term. It is now well established that the biological actions of a hormone can be effectively neutralized through the introduction of a high-affinity antibody through passive immunization (Scaramuzzi, 1975). This technique was chosen in application to diminishing estrogen levels because most synthetic estrogen antagonists are inherently estrogenic themselves as they function as receptor blockers (Emmens et al., 1967).

Subjects and Methods

Female HS mice were housed and inseminated following the previously described procedures. Five groups of subjects were prepared which consisted of an untreated control group, a vehicle injection control group (NRS), and three doses of anti-estrogen. Since estrogen is known to be essential for implantation, a dose of antibody was chosen which would bind with less than 50% of the free endogenous hormone to avoid depleting natural estrogen completely. The antibody used was donated by Dr. E.V. Younglai and had been prepared by injecting rabbits with estrogen. A preliminary experiment aimed at assessing the relative potency of the antibody in vivo utilized a 1:10 of AB:saline solution. This pilot study yielded significant differences between the control group and

the AB and NRS groups in terms of number of pups born ($F(2, 35)=6.56$, $p=0.004$), number surviving to day 3 ($F(2, 35)=7.46$, $p=0.002$), and litter weight on day 3 ($F(2,35)=7.04$, $p=0.003$). Duncan's multiple comparisons ($p < .05$) were conducted for all of these measures, which indicated that the control group differed from both the NRS and AB groups, but no differences existed between these two. The percent of females bearing litters, percent of pups cannibalized, and percent of pups stillborn for all conditions were also calculated. These results indicated that there was a trend in the percent of successful pregnancies with the AB group having 54% dams delivering litters and only 38% of the NRS group delivering as compared to the control group which showed a pregnancy rate of 100%. There were no differences in terms of number of stillborn pups or number of cannibalizations. Thus, it was apparent that the chosen dose may have been insufficient.

A binding assay was done on the antibody and revealed that a 1:250 dilution resulted in 50% binding in vitro. Consequently, the following doses were utilized in this experiment: 1:10, 1:50; 1:100 antibody:saline. All injections were administered daily immediately before placement in the restraint device as described earlier. The injections and restraint regimen again began on day one of pregnancy and continued through day five. At the end of the restraint period on day 5, animals were anesthetized with .03 ml sodium pentobarbitol. Cesarean sections were then performed by extending an incision dorsally from the mid abdominal region to just above the vaginal opening. Uteri were removed, dissected free of fat, and the number of implantation sites for each animal was recorded. This method was chosen rather than the

measurement of actual number of pups born because of possible unknown long term effects of the antibody.

Results

An analysis of variance on the mean number of implantation sites per group was marginally significant, $F(4,95)=2.49$, $p=0.05$, however multiple comparisons showed that although there was a significant difference between the mean number of implantation sites in the control group as compared to the vehicle group ($p < .01$), the antibody groups were not significantly different from each other or from the control or vehicle groups. A chi square analysis of association between presence or absence of implantation sites and experimental conditions failed to suggest rejection of the hypothesis that pregnancy outcome was unrelated to condition, $X^2(4)=8.09$, $p<.10$. Table 15 and Figure 5 show the results for this experiment. Since no significant difference existed between any of the antibody groups, the data from these animals were collapsed and analyzed as one antibody group in comparison to the control and vehicle groups. These data are illustrated in Figure 6. This analysis proved to be significant through analysis of variance, $F(2,97)=5.06$, $p=0.008$, and Chi square analysis suggested a rejection of the hypothesis that presence or absence of implantation sites was unrelated to conditions, $X^2(2)=7.48$, $p<.025$. Again, multiple comparisons indicated that the vehicle group differed significantly from the controls ($p < .01$), but the antibody group failed to differ from either of the other two. A trend was apparent in the percent of animals having

Table 15

Sample Size, Percent of Females Having Implantation Sites, and Mean (± S.E.) Number of Implantation Sites for Each Antibody (AB) Dose, for the Vehicle (NRS), and Control Conditions in Experiment 7

Group	n	% Imp. Sites	# Imp. Sites
restraint + vehicle	20	50	4.1 ± 1.3
restraint + 1:10 AB:NRS	20	70	6.8 ± 1.3
restraint + 1:50 AB:NRS	20	60	6.3 ± 1.4
restraint + 1:100 AB:NRS	20	70	7.3 ± 1.3
Control	20	90	9.5 ± 1.0

implantation sites in that 90% of the controls had implantation sites while only 50% of the stressed dams receiving the vehicle had implantation sites. The lowest AB dose (1:100 AB:NRS) resulted in implantations in 70% of mothers as did the highest dose (1:10 AB:NRS). The middle dose (1:50 AB:NRS) resulted in only 60% of mothers possessing implantation sites, however this difference was not significant.

Figure 5: The mean (±SE) number of implantation sites present after exposure to physical restraint and treatment with varied daily doses of an antibody to estrogen or vehicle in Experiment 7.

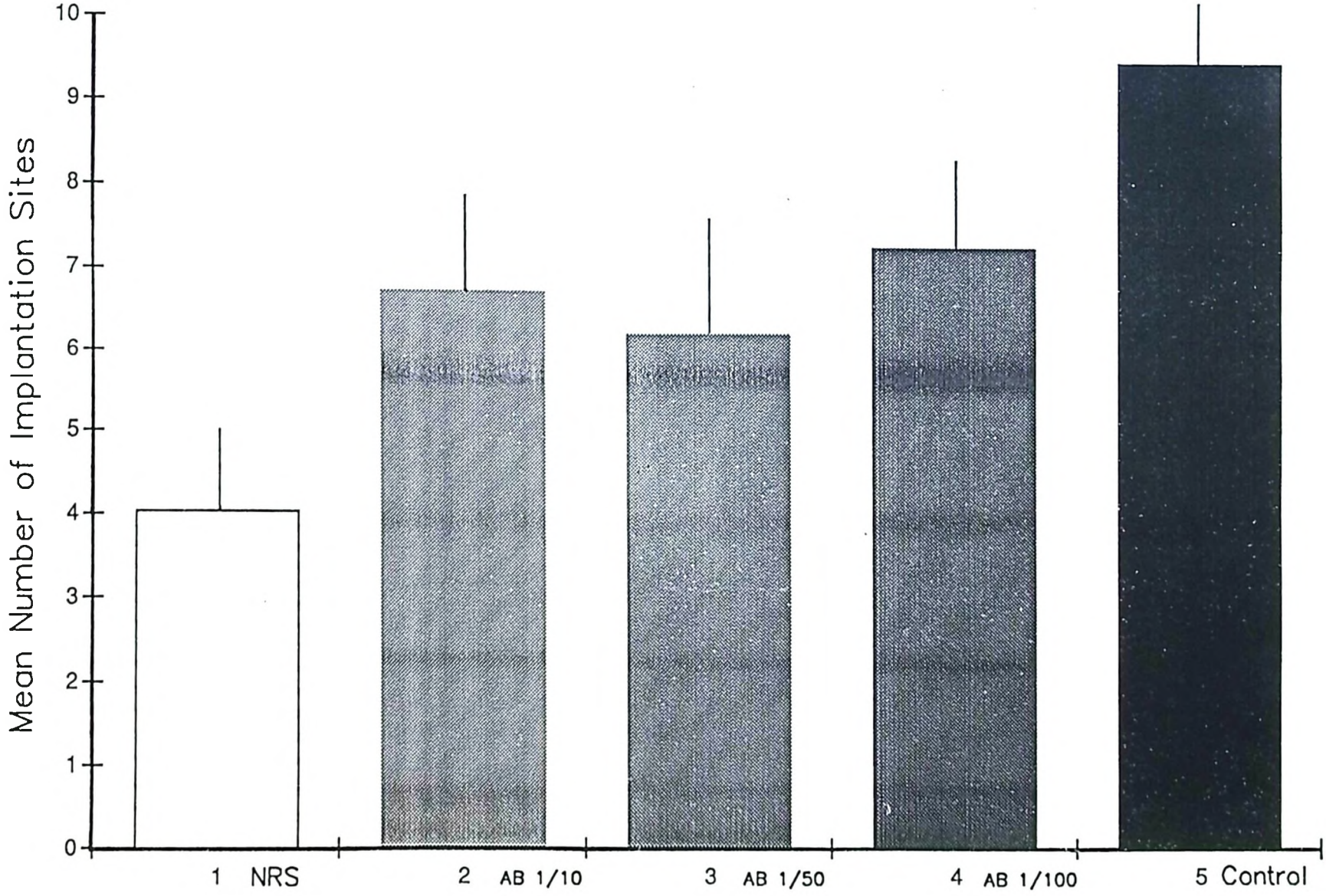
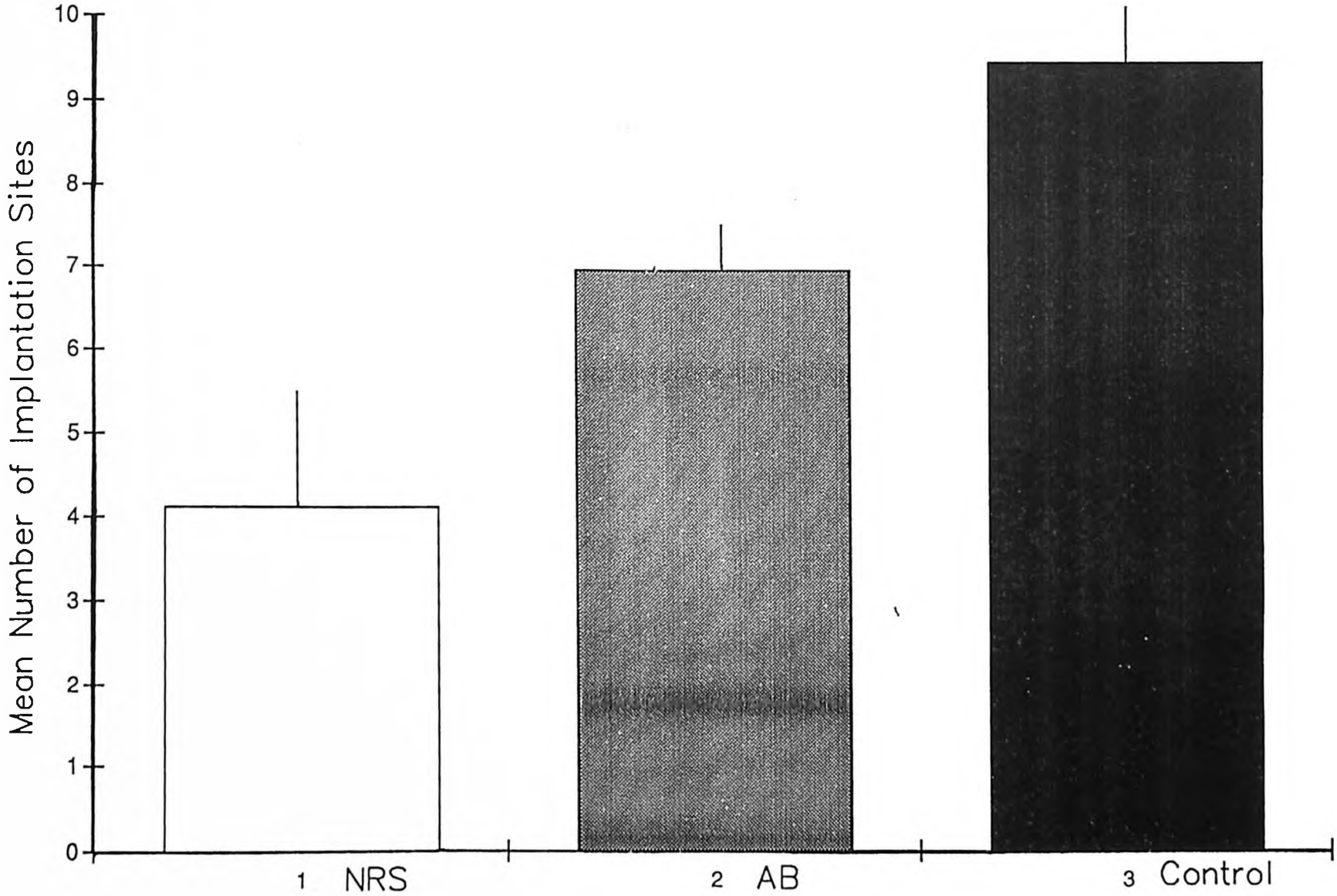

Mean Number of Implantation Sites
10
9
8
7
6
5
4
3
2
1
0
1 NRS
2 AB 1/10
3 AB 1/50
4 AB 1/100
5 Control

Figure 6: The mean (±SE) number of implantation sites after exposure to physical restraint and treatment with an antibody to estrogen (data collapsed across doses) or vehicle in Experiment 7.


Mean Number of Implantation Sites
10
9
8
7
6
5
4
3
2
1
0
1 NRS
2 AB
3 Control

Discussion

These results suggest that exogenous administration of an antibody to estrogen may be partially effective in protecting pregnancy against the deleterious effects of stress. A more thorough dose response curve should be done in order to determine the optimal dose of antibody as none of those used here was significantly effective on its own. Furthermore, as there was much variance in the day 1 weights of the subjects, it is possible that a dose per body weight treatment regimen may be more effective than a global dose. It is also possible that a littering out strategy may have yielded a greater difference between the NRS and antibody groups as both Euker & Riegle (1973) and Wiebold et al. (1986) reported that there are long term effects of stress administered in the first days of pregnancy which are not apparent until after day 7. Furthermore, the NRS was obtained from a female rabbit and thus there may have been enough endogenous progesterone in the serum to provide some protection against stressor administration in early pregnancy.

It is not surprising that the estrogen antibody was less potent in reinstating pregnancy than progesterone. First, it is unlikely that a synthetic intervention would be as effective in "righting" biological homeostasis as a natural treatment since the administration of a foreign substance could result in a second stress-like response. Furthermore, although the antibody was successful in partially suppressing enhanced estrogen levels, endogenous progesterone levels were also likely affected by stress, and it is probable that the antibody was not capable of reinstating the critical ratio necessary for implantation.

CHAPTER SIX

DIRECT ANALYSIS OF THE HORMONAL CORRELATES OF STRESS IN EARLY PREGNANCY

Experiment 8

Introduction

It is obvious that the experiments presented thus far have been indirect indicators of the influence of stress on progesterone and estrogen. In order to assess these effects confidently, and to establish the role of ovarian hormonal mechanisms in stress-induced pregnancy blocks, direct measures of hormone levels under stressful conditions were assessed via radioimmunoassays. Rats were used in this experiment because the large amount of plasma necessary to determine estrogen alone was more than can be obtained from one animal. Rather than introducing confounds incurred by pooling the blood of several animals, a large quantity of blood was obtained from each subject sufficient for all three assays from one animal so that the relationships between these hormones could be more accurately examined.

Subjects

Subjects were 100 female Long-Evans hooded rats bred in our department. Animals were housed in groups of 3 in standard polypropylene cages with wire grid tops allowing continuous access to food and water. The colony room was maintained under a reversed 10:14 hr dark/light cycle and at 21 ± 1 °C. Isolated males of the same strain were also housed under these conditions.

Methods

Subjects were inseminated as previously described for mice, and were then randomly assigned to either control or stress groups.

Experimental animals were subjected to 5 hours of restraint on each of days 1 to 5 after insemination. Restraint was achieved by allowing the animals to enter a plastic tunnel and then blocking retreat. This particular method of immobilization has also been found to be effective in producing the standard physiological indices of stress (Pare & Glavin, 1986). Control animals were simply placed alone in clean cages and remained undisturbed until day of sacrifice.

Ten experimental animals were anesthetized with sodium pentobarbitol (60mg/kg) on each of the first five days of pregnancy immediately following completion of the restraint period. Ten control animals were also anesthetized at this time. Animals were then bled out via open heart puncture with heparinized syringes. This procedure was chosen over decapitation or a blow to the skull due to the large quantity

of blood needed from each animal. Blood was then centrifuged at 3000 rpm for ten minutes. The blood of each animal was frozen separately in two equal aliquots at -65°C until assay. The samples were assayed together for corticosterone, progesterone and estrogen. The exact assay procedure is described in Appendix 1. A number of preliminary assays were completed in order to apply the existing procedure to rat blood. This procedure is described in detail in Appendix 2.

Results

Table 16 shows the mean levels of all three hormones. As shown in Figure 7, the mean corticosterone levels (ug/ml) were elevated on each day after insemination for the experimental group. Analyses of variance performed on the percent binding obtained indicated that the experimental group exhibited a significantly higher level of corticosterone than the controls, $F(1,90)=15.55$, $p<=0.001$. Surprisingly, the mean progesterone level was also elevated in the restrained animals, as compared to controls, on each day of pregnancy, except for the fifth $F(1,90)=8.44$, $p=0.005$. Figure 8 shows the mean levels of progesterone on each day after insemination for both conditions in ng/ml. Figure 9 shows the mean 17-β estradiol levels on each day after insemination for both conditions in pg/ml.. Analyses of variance performed on the percent binding values obtained revealed that estradiol levels were significantly higher in the stressed group, $F(1,90)=4.71$, $p=0.031$, and were elevated for each day but the fifth. Analyses of variance using the

Table 16

Mean Levels of Corticosterone, Estrogen and Progesterone in Stressed and Control Animals During the First Five Days of Pregnancy

Day	Group	Corticosterone (ug/100 ml)	Estrogen (pg/ml)	Progesterone (ng/ml)
1	Stress	224.496	28.58	33.90
2		272.86	29.92	46.56
3		247.09	32.11	58.00
4		293.30	38.93	57.89
5		246.75	25.36	47.64
1	Control	200.98	27.24	27.32
2		142.25	23.05	33.70
3		142.64	27.07	37.95
4		122.54	26.10	46.62
5		237.44	31.02	55.55

Figure 7: Mean (±SE) Plasma Corticosterone Levels in Stressed and Control Animals Over The First Five Days of Pregnancy.

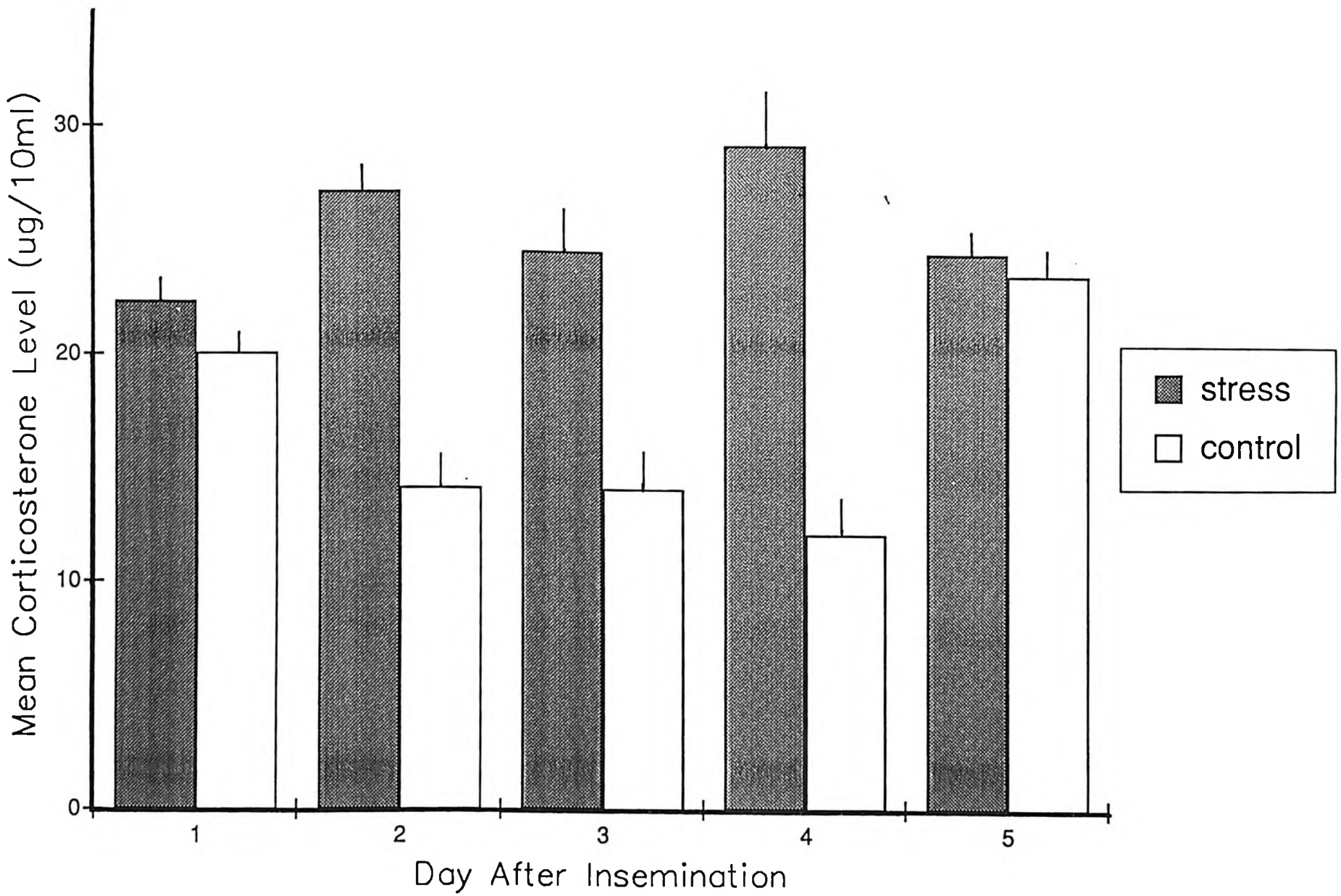

Mean Corticosterone Level (ug/10ml)
30
20
10
0
1
2
3
4
5
Day After Insemination
stress
control

Figure 8: Mean (±SE) Plasma Progesterone Levels in Stressed and Control Animals Over The First Five Days of Pregnancy.

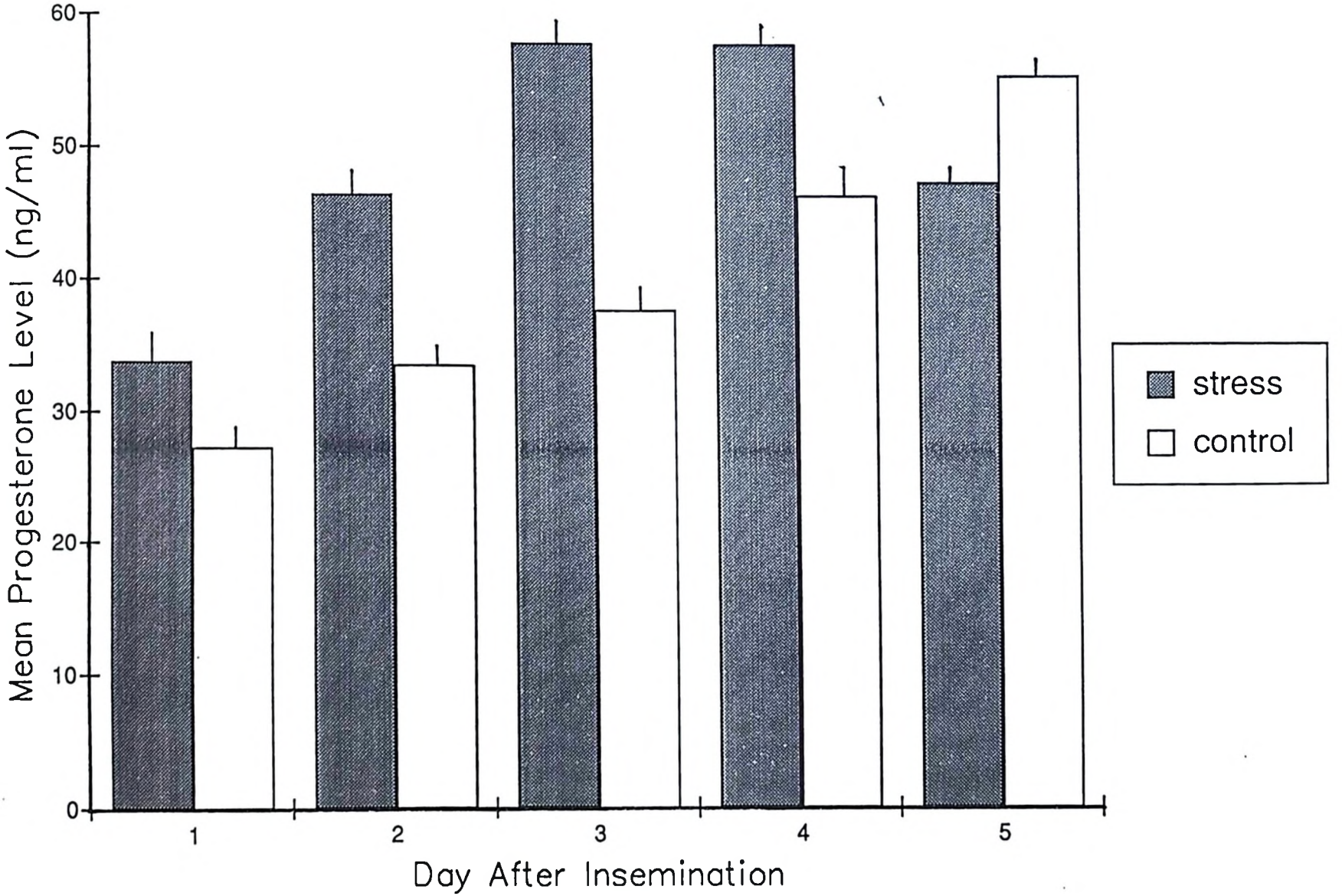
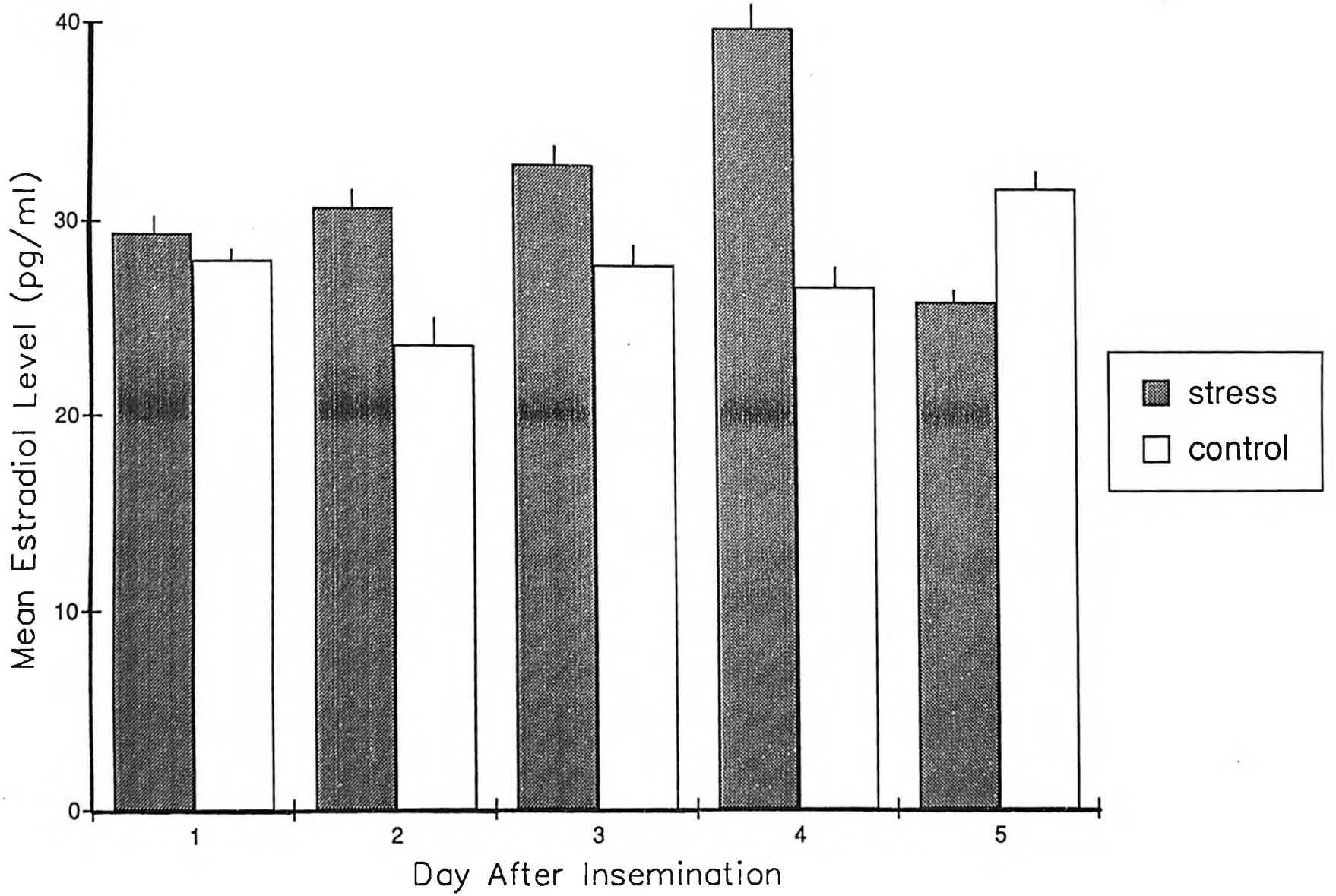

60
50
40
30
20
10
0
Mean Progesterone Level (ng/ml)
1
2
3
4
5
Day After Insemination
stress
control

Figure 9: Mean (±SE) Plasma Estradiol Levels in Stressed and Control Animals Over The First Five Days of Pregnancy.


40
30
20
10
0
Mean Estradiol Level (pg/ml)
1
2
3
4
5
Day After Insemination
stress
control

exact counts obtained also yielded these significant results. The values obtained for the control animals were in accordance with those previously reported (Watson, Anderson, Alam, O'Grady and Heald, 1975), although some disparity exists due to variables such as differences in the strain of rats used, the time of day of sampling, the assay procedure followed and the use of anesthetic. As all samples were assayed in a single assay, there was no interassay variance. The intra-assay variance was 1.8% for corticosterone, 3.7% for progesterone and 0.9% for estrogen. Multiple comparisons using Duncan's Test revealed that the stress group differed from the controls on days 2 ($p<.05$) and 4 ($p<.01$) for corticosterone, on day 3 for progesterone ($p<.05$) and on day 4 for estrogen ($p<.05$). Due to the large quantity of blood needed for the estrogen assay, only the progesterone assay could be run twice and the results were replicated.

Discussion

As expected, corticosterone levels in the stressed group of animals were higher than in controls. This is consistent with past reports that restraint stress elevates corticosterone in rats (Reigle, 1973) and mice (Barlow et al., 1974; Barlow et al.. 1975).

The increase in progesterone levels following restraint stress is consistent with previous findings that both adrenal and ovarian output of progesterone are enhanced during stress exposure (Fenske, 1986; Fajer et al., 1971; Fuller et al., 1984; Holzbauer & Newport, 1967; Miller and Riegle, 1985; Pollard and Dyer, 1981; Vincent, 1971) and after ACTH

administration (Piva et al., 1977; Resko, 1969). On the other hand, there are reports which suggest that progesterone levels can decrease during chronic stress exposure (Albrecht et al., 1978; Wiebold et al., 1986). It is possible that stress may cause both a decrease and an increase in progesterone depending on the time at which progesterone is measured. For example, one study showed that heat stress applied in early pregnancy caused an enhancement of progesterone levels in swine, however a decrease in progesterone levels was observed in response to stress applied in late pregnancy (Wetteman & Bazer, 1985). Another experiment indicated that ACTH infusion over the estrous cycle in heifers caused an increase in progesterone for the first five days followed by a decrease for the last three days, while corticosterone levels remained elevated throughout (Wagner, Strohben & Harris, 1972).

Endogenous estrogen levels were also higher in the animals exposed to stress than in controls. Although the effects of stress on estrogen in pregnancy have not previously been reported, these results are supported by studies suggesting that ACTH administration can cause increased estrogen levels in nonpregnant (Arai et al, 1972) and pregnant human subjects (Simmer et al., 1983; Strott et al., 1975).

The finding that both estrogen and progesterone were lower in the stressed animals than the controls on day five suggests that the stressed animals were no longer pregnant by this time which is consistent with the effects of stress on implantation.

Although it may seem that the stress induced increase in progesterone observed here is inconsistent with the results of experiments 2 and 3, it is possible that exogenous progesterone

administration could aid in the maintenance of pregnancy despite the fact that progesterone was not depleted. Porter (1970) reported that exogenous progesterone was partly effective in preventing pregnancy loss due to estrogen administration. Furthermore, Harper (1967) reported that progesterone would protect pregnancy against the harmful effects of androstenedione. Given that there is an inhibition of uterine receptivity when the progesterone to estrogen ratio is disturbed (McLaren, 1960), it is possible that the stress induced pregnancy loss occurs due to toxic levels of estrogen produced by stress, and that progesterone may be somewhat effective in counteracting this effect.

Given that all three hormones increased due to restraint, it is possible that external stress has a global stimulatory effect on steroid output, perhaps under ACTH modulation. Indeed, Fenske (1986) noted that there was a positive correlation between progesterone and corticosteroid release in response to a stimulus. Other previous reports indicate effects consistent with an increase of gonadal hormone output under stress. Weir & De Fries (1963) found that after stress, these animals were more likely to conceive than controls. They took this to suggest that there was a possible enhancement of gonadal hormones under these circumstances. Similarly, Zondek & Tamari (1960) observed that contrary to Selye's (1936) report of the atrophy of gonads under stress, stressed non pregnant animals actually had heavier ovaries and more corpora lutea than controls again suggesting a release, rather than a depletion, of ovarian hormones. Support for this notion comes from studies which suggest that estrogen administration will increase ovarian weight (Harper, 1967, 1969).

As shown in Experiment 5, endogenous 17-β estradiol, on the order of 0.333 micrograms/day s.c. over 5 days prior to, and around the time of implantation, will completely block pregnancy in all female mice. Similarly, Experiment 6 confirmed and extended previous reports that diverse artificial estrogens also block early pregnancy (Chang & Yanagimachi, 1965; Greenwald, 1961; Whitney & Burdick, 1936). The present data show that endogenous estrogen is increased by chronic stress in early pregnancy. Furthermore, administration of an estrogen antibody was partially effective in reversing a stress induced pregnancy block in mice. The finding that endogenous estrogen increases under chronic stress may account for pregnancy failures associated with diverse psychological and physiological stressors.

Future research should examine in fine detail the changes in the secretion of these hormones in response to the application of a stressor. It would not be difficult to withdraw small samples of blood from the tail vein of restrained animals at short intervals over the course of the stress procedure. It would be necessary to pool the blood of many animals, however, as sufficient quantities of plasma for the estrogen assay could not be obtained from one animal in this manner. It is possible that stress could first cause a rise in steroidal output that may reach a high point and be maintained for some time. Steroid levels may then drop either due to habituation of the animal to the stressor or to a failure of the adrenal gland to maintain such a high output. Hormone levels could then remain at a low level, or they may rebound to the former high level. It would also be interesting to examine the hormone levels of animals who fail to abort in response to stress and instead are

able to continue to term despite their adverse conditions. It is possible that some genetic variable may predispose these subjects to behavioural coping mechanisms or to a different hormone profile which may aid in combatting the effects of stress.

Another line of study could attempt to localize the site of stress action. For example, ACTH may cause estrogen secretion from either the adrenal, the ovaries or both. Also, pituitary stimulation in response to a stressor may result in gonadotropin release which may in turn stimulate estrogen and progesterone secretion. One approach to this issue could involve surgical manipulations at each physiological level such as ovariectomy to remove gonadal contribution. Furthermore, ovariectomy followed by the administration of exogenous hormones under stress could be done to determine the exact ratio of hormones necessary to maintain pregnancy despite adverse conditions.

It has been demonstrated herein that stress exposure shortly after mating can cause a disruption of the fine progesterone:estrogen balance critical for the establishment and maintenance of pregnancy. This focus on the role of ovarian steroids in stress-induced pregnancy disruptions is unique, and may indeed be the mechanism underlying this effect. However, the pattern of physiological responses to a stressor is complex, and the effects of this sustained arousal are varied. Consequently, although it is conceivable that small stress-induced enhancements of endogenous estrogens may account for the unexplained adverse effects of stress on pregnancy, it is likely that this mechanism does not work in isolation. Further quantitative analyses are necessary

to evaluate the adequacy of this hypothesis, and to examine the interactions with other biochemical systems.

Appendix 1

Radioimmunassays (RIAs)

The basic principle involved in the RIAs is one of competitive binding. Here, a commercially produced radioactive antigen of the hormone in question competes with the non-radioactive version of the same antigen for a fixed number of specific antibody binding sites. When the unlabelled antigen from standards or samples, and a fixed amount of the labelled antigen are allowed to react with a constant and limiting amount of antibody, decreasing amounts of sample antigen are bound to the antibody as the amount of labelled antigen increases. Thus, the amount of hormone present in a sample can be calculated based on the amount of radioactivity measured in the sample by a beta counter. The lowest counts will occur in the tube the highest original concentration of radioligand. The greater the concentration of native hormone, the lower the count because less radioligand is able to bind to the receptor.

Assay Procedure

1. Extraction

Progesterone, estrogen and corticosterone are extracted from the serum using diethyl ether in the following manner.

1. Aliquots of serum are pipetted in duplicate into extraction tubes to which 3ml of diethyl ether is added.
2. The tubes are then vortexed gently twice and allowed to sit in the fumehood for 15 minutes.
3. After this time, the ether supernatant is pipetted off and placed in 12x75mm assay tubes. These tubes are then placed in a warm water bath and blown down under air.
4. Steps 1-3 are repeated. 100 ul of buffer are then added to the dried down extracts. The tubes are then allowed to sit in the water bath for 1/2 hour and are then covered with parafilm and placed in the coldroom overnight.
5. The next day, standards containing known amounts of hormone, as well as a zero and a nonspecific binding (NSB) tube are dried down under in the waterbath.
6. 100 ul of buffer are then added to these tubes and they are vortexed.
7. 100 ul of the appropriate antisera are added to all tubes, including the extracted unknowns.
8. All tubes are then vortexed and allowed to sit for 30 minutes.
9. 100 ul of the appropriate radiolabelled hormone are added to the tubes.

10. The tubes are then vortexed, covered with parafilm and placed in the coldroom overnight.
11. The next day, 1 ml of charcoal-dextran solution is added to the tubes.
12. Tubes are vortexed and allowed to sit in the coldroom for 10 minutes.
13. Tubes are then centrifuged at 3000 rpm for 10 minutes.
14. The supernatant of each tube is then decanted into 6 ml of scintillation fluid.
15. Vials are then shaken vigorously and allowed to sit in the beta counter for 1 hour.
16. Samples are then counted twice for 1 minute each.

Calculations:

1. The counts per minute (cpm) obtained for the nonspecific binding tube (NSB) are subtracted from the cpm from the zero tube.
2. The percent binding of the standards are calculated according to the following formula:

$$\frac{\text{standard cpm - NSB cpm}}{\text{(zero - NSB cpm) x 100}}$$

3. A standard curve is then plotted on semi-log paper with the amount of progesterone on the ordinate axis and the counts per minute on the abcissa.
4. Percent binding of the unknowns are then calculated using the same method as for the standards. The quantity of progesterone present is then determined by locating the point on the standard curve corresponding to the obtained percent binding, and reading the associated progesterone level from the ordinate.

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