

قسم الفسيولوجيا والكيمياء الحيوية والأقربازين  
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## تأثير الاجهاد على التغيرات المجهريّة والكيميائية الحيويّة التالفة لاحداث التقرص المشيمي في أرحام الفئران

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أحدث التقرص المشيمي في بطانة الرحم لأحد قرني الرحم وذلك بتقسيط زيت الزيتون في أرحام فئران مستأصل منها المبايض ومعالجة بالهرمونات المناسبة • كانت قرون الرحم المحدث بها التقرص المشيمي أكثر وزناً وتحتوي على مستوى مرتفع معنوياً من البروتين الكلي ومن نشاط أنزيمي الفوسفاتيز القلوي والحامضي بالمقارنة بالقرون الحكم •

نتج عن تعريض مجموعة من الفئران الى الاجهاد بتقييد حركتها ٤ ساعات يومياً لمدة أربعة أيام نقصاً معنوياً في أوزان هذه الفئران ونقصاً في البروتين ونشاط أنزيمي الفوسفاتيز القلوي والحامضي في المصل وكذلك استنفاد محتوى الغدة الكظرية من الكوليستيرول • كذلك قلل الاجهاد من استجابة القرون الرحمية الى التقرص المشيمي والتي أصبحت أصغر كثيراً من تلك الموجودة في أرحام الفئران الغير مجهدة • كذلك قلل الاجهاد من عدد الغدد الرحمية وعدد الأوعية الدموية في الأقرص المشيمية •

يقترح البحث الحالي أن تعريض الحيوانات في بداية فترة الحمل الى عوامل الاجهاد قد يقلل أو حتى يتعارض مع تكوين الأقرص المشيمية في الرحم •



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**STRESS EFFECTS ON MORPHOLOGICAL AND BIOCHEMICAL CHANGES  
FOLLOWING INDUCED DECIDUALIZATION IN RAT UTERI**

(With 3 Tables & 5 Figures)

By

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**SUMMARY**

Unilateral decidualization was induced in the endometrium by non-surgical instillation of oil in uteri of spayed rats after proper hormonal treatment. Decidualized uteri were heavier in weight, had significantly higher levels of total protein, alkaline phosphatase (ALP) and acid phosphatase activities as compared to non decidualized horns. Immobilization stress (4h/Day for 4 days) significantly reduced body weights, total serum proteins, serum ALP, serum acid phosphatase and depleted the adrenal cholesterol content. Also, stress reduced the intensity of the decidual cell reaction (DCR) in the decidualized horns which had fewer uterine glands, reduced vascularization and smaller decidual cell growth.

It is suggested that stressing of early pregnant animals may delay or even interfere with the DCR in their uteri.

**INTRODUCTION**

A known fact in animal reproduction is the lowered fertility of wild animals kept in captivity and subjected to restraint stress. As shown by BONFILS and LAMBING (1963), restraint immobilization is a form of psychological stress because of the threat it represents for the restrained animal. Stress during pregnancy has adverse effects on reproductive function in laboratory rodents. Auditory stimuli at regular intervals for 48h appeared to interfere with the implantation process in rats (ZONDEK and TAMARI, 1967). Restraint stress for 2h on each of the first five days of pregnancy can decrease average litter size in rats (EUKER and RIEGLE, 1973). Moreover, stressing of cyclic female rats during the afternoon of pro-oestrus blocks ovulation (HULSE; COLEMAN; NICHOLAS and GREENWOOD, 1982) and increases serum prolactin levels (JAHN and DEIS, 1986). A recent and interesting study by WIEBOLD; STANFIELD; BECKER and HILLERS (1986) showed that restraint stress for 5h on the first 6 Days of pregnancy reduced the pregnancy rate and average litter size of mice.

This investigation was initiated to determine the significance and mode of action of stress as a potentiating factor in affecting the decidual cell reaction (DCR) in the endometrium and consequently its probable effect on the implantation process. The model of induced decidualization was used in the present study to mimic early pregnancy (FINN and MARTIN, 1972).



### MATERIAL and METHODS

Female rats (Alexandria High Health Institute strain) were ovariectomized. They weighed 250-270g. at the start of experiments. Two weeks after ovariectomy, the rats were started on a hormone regimen developed FINN (1971) for maximum sensitization of the uterus to the decidual stimulus. Text-figure 1 outlines the schedule of this procedure. Non-surgical intra uterine injection of olive oil was used to induce artificial decidualization of the endometrium. The oil (about 50  $\mu$ l) was injected randomly into either horn using a plastic cannula (0.5 mm inner and 1 mm outer diameter) connected to a 1 ml plastic syringe. The animal was lightly anaesthetized with ether and the cannula was inserted through the cervical canal after visualization of the cervix through a condensed light. At the time of oil instillation it was not known which animals would be subjected to stress. The Day of unilateral oil injection was considered Day 1 of pseudopregnancy. Animals were divided into two equal groups each of six rats. The first group was kept as unstressed control group. Rats in the second group were subjected to immobilization stress. Each rat was strapped on its back for 4h/Day for 4 Days. Uterine responsiveness to the decidual stimulus was maintained in all rats by subcutaneous injection of 3 mg progesterone plus 200ng oestradiol-17 $\beta$  in olive oil/Day. On Day 4 of pseudopregnancy, rats in the unstressed group and those subjected to stress (after their last exposure to immobilization stress) were weighed to the nearest g then killed. Blood samples were collected from the trunk into clean centrifuge tubes fitted with funnels. After coagulation, serum samples were separated and stored/frozen until used for biochemical analysis. The adrenal glands were dissected out, weighed to the nearest mg, then stored frozen. The uterine horns were transected just above the cervix and dissected free from fat and weighed separately to the nearest mg. The magnitude of the decidual reaction was assumed to be proportional to the weight of the horn (FINN, 1971). Uterine segments were taken from each horn for histological evaluation and the remainder of each horn was immediately frozen for biochemical determinations. For histological studies, uterine specimens were fixed in 10% formalin and processed routinely for embedding in paraffin wax and sectioned at 5 $\mu$ m. The sections were stained with H&E and PAS. Total proteins in sera and uterine tissues were determined by the Biuret method cited in WOOTON (1964). The optical density was measured at 540 nm. in a spekol colorimeter. The activities of alkaline and acid phosphatases in sera and uterine tissues were estimated by the disodium phenyl phosphate method of KIND and KING (1954). Optical density was measured at 510 nm. and the enzyme activities were expressed as the production of 1 mg. of phenol in 15 minutes under the conditions of the test. Statistical analysis was run by Student t test.

### RESULTS

Rats subjected to immobilization stress applied for 4h/Day for 4 Days following the induction of unilateral uterine decidualization, showed significant loss in their body weights ( $P/0.005$ ), had significantly heavier adrenal glands ( $P/0.005$ ) which were lower in their cholesterol content ( $P/0.001$ ) than those of unstressed rats.

Levels of serum total proteins, alkaline phosphatase (ALP) and acid phosphatase activities were significantly reduced in stressed rats ( $P/0.005$ ;  $P/0.05$  and  $P/0.02$  respectively) as compared to levels in unstressed rats. The decidualized uterine horns were much heavier than the non decidualized horns of the same animal both in control ( $P/0.001$ ) and in stressed rats ( $P/0.05$ ). However, the percentage gain in weights of the decidualized horns of the control unstressed rats was significantly higher than that in stressed animals ( $P/0.005$ ). The decidualized



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uterine horns had higher levels of total protein (mg%) ( $P/0.001$ ) and ALP activities ( $P/0.001$ ) over those of the non-decidualized horns irrespective to stress application. Acid phosphatase activity was significantly increased ( $P/0.005$ ) in the decidualized horns of stressed but not in the unstressed rats.

Microscopical examination of histological sections taken from the uterine horns revealed the following main findings:

### 1- Non decidualized horns of control unstressed rats:

The endometrium have thick mucosal folds which were richly vascularized and protruded into the crescentic shaped lumen (commonly found opposite to the mesometrial attachment). There was no evidence of a decidual cell reaction in the stromal cells (Fig. 2).

### 2- Decidualized uterine horns of unstressed rats:

A distinct decidualized stromal cell reaction was evident. The deciduomatus cell growth completely occluded the uterine lumen and distended its wall. Many mitotic figures were observed in the stromal cells. An extensive vascularization was seen with the appearance of a broad connective tissue vascular zone inbetween the two layers of the myometrium. The lamina epithelialis was absent. (Fig. 3).

### 3- Non decidualized horns of stressed rats:

The cross diameter and thickness of the uterine wall were much reduced as compared to their respective in the unstressed rats. The mucosal folds of the endometrium were fewer and shorter than in the non decidualized horns of the unstressed rats. The stroma was highly vascularized with clear oedema specially opposite to the mesometrial attachment. No DCR was observed. The uterine glands were less developed and the myometrium was much reduced in thickness to a very thin muscular layer. (Fig. 4).

### 4- Decidualized horns of stressed rats:

The DCR was clear but less in magnitude than that observed in the decidualized horns of unstressed rats. The deciduomatous growth did not occlude the uterine lumen. Eccentric foci of eosinophilic cells were seen. The uterine glands were rarely observed near the inner muscular layer. The thickness of the muscular layers and the vascular layer inbetween was greatly reduced in comparison to those of unstressed rats. (Fig. 5).

## DISCUSSION

Stress has adverse effects on reproductive function in laboratory rodents. Stressing of cyclic female rats during the afternoon of pro-estrus blocks the luteinizing hormone (LH) surge and ultimately ovulation (KRIEG; STEE; LAMBERTS & MCLEOD, 1978; HULSE, *et al.* 1982). Also, restraint stress in early pregnancy in mice reduced the pregnancy rate and average litter size (WIEBOLD, *et al.* 1986). In the present study, immobilization stress of pseudopregnant rats for 4h/Day for 4 Days significantly reduced the body weights and total serum proteins of stressed rats. Body weight is highly correlated with litter size (ELLIOT; LEGATES & ULBERG, 1968; BAKKER; WALLINGS & POLITICK, 1978). Besides, the present study showed a highly significant decrease in weights of decidualized horns of stressed rats as compared with weights of unstressed control rats. Moreover, examination of histological sections prepared from the uterine horns showed that, as compared to the decidualized horns of unstressed rats, those of stressed rats



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were much reduced in cross diameter, had much fewer uterine glands, had smaller decidualoma-tous cell growth which did not occlude the uterine lumen and had much less extensive vascular layer. Increased vascularization and increased vascular permeability are the most prominent features of decidualization in the uterus (KENNEDY, 1980 and MILLIGAN & MIREMBE, 1984) and inhibition of these vascular reactions at implantation sites would interfere with implantation process (KENNEDY, 1977; EVANS & KENNEDY, 1978).

Stress-induced changes in ACTH and corticosteroids cannot be overlooked when discussing the adverse effects of restraint stress on pregnancy. Restraint is an effective means of activating the hypothalamic pituitary-adrenal axis (RIEGLE, 1973) and immobilization stress used in this study (restraint on a wire-mesh frame) has been shown to raise plasma corticosterone concentrations (and presumably ACTH) 3-fold over those in control mice (BLECHA; KELLY & SATTERLEE, 1982). Both ACTH and adrenal corticoids have been shown to reduce the litter size in rats and this effect appeared to be mediated through the adrenal glands (VELARDO, 1957). In the present study, the adrenal glands of stressed rats were hypertrophied and depleted from its cholesterol stores suggesting increased production of adrenal steroids probably due to stress-induced over production of ACTH.

Alkaline phosphatase enzyme plays an important role in transferring metabolites across the cell membrane. The increased ALP activity in the stroma of the uterus during early pregnancy is already well documented in mice (SMITH, 1973; MURDOCH, KAY & CROSS, 1978), rat (JELINCK and JELINKOVA, 1975) and rabbit (MURDOCH, 1972). The results of the present investigation using biochemical assay techniques confirm these observations and demonstrate a significant increase in ALP activity in the decidualized uterine horns over the non-decidualized horns. The factors responsible for the regulation of ALP activity in the decidua during pregnancy and pseudopregnancy remain to be elucidated. The present results showed that immobilization stress significantly reduced serum but not uterine levels of ALP. A further study in which stress is applied over a longer period of pseudopregnancy would be helpful since it was reported that maximum increases in uterine ALP activity occurred between Days 5 and 7 of pseudopregnancy (MURDOCH, et al. 1978).

Acid phosphatase, which has been shown to increase on Day 6 of pregnancy (parathasara-thy, Purandare, Katarak, JUNEJA & MUNSHI, 1979) is associated with the breakdown and phago-cytosis of luminal epithelial cells at the site of implantation (MALINOWSK & FOTHERBY, 1975). In the present study, the disappearance of the luminal epithelium observed in decidualized horns of unstressed rats—as evidenced by histological examination—was associated with a significant increase in serum—but again—not uterine levels of acid phosphatase activity.

It therefore may be concluded that restraint stress during early stages of pregnancy, at peri-implantation times, may delay or even interfere with the formation of a well developed decidualoma and ultimately inhibit implantation, a mechanism which is added to the previously reported (HALSE, et al. 1982) stress induced inhibition of ovulation.

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Table (1)  
Body weight (b.w.), uterine weight, % gain in uterine wt., adrenal weights and adrenal cholesterol content of pseudopregnant unstressed and stressed rats

	Body weight	Uterine weights (mg/100g b.w.) Non-decid. horns.	Decidualized horns	% gain in wt. of uterine horns	Adrenal wt. (mg/100g b.w.)	Adrenal cholesterol (mg/mg tissue)
Control group	266.6±8.41 (6)	0.137 ± 0.03 (5)	0.358±0.03 <sup>d</sup> (7)	161.4 ± 26.1	0.02±0.001 (6)	0.04 ±0.006
Stressed rats	195.4±8.96 <sup>a</sup> (5)	0.073 ± 0.00 <sup>c</sup> (4)	0.130±0.02 <sup>a,e</sup> (6)	54.5 ± 16.9 <sup>b</sup>	0.03±0.003 <sup>b</sup> (5)	0.0064±0.001 <sup>a</sup>

- Values are means±s.e. and are significantly different from control values at: a (P/ 0.001); b (P/ 0.005) and c (P/ 0.02).
- Significantly different from the corresponding non-decidualized horns at: d (P/ 0.001) & e (P/ 0.02).
- Decidualization was induced in spayed hormonally sensitized rats by unilateral instillation of olive oil in one horn.



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Table (2)  
Effect of stress on total proteins, alkaline phosphatase and acid phosphatase levels in rat uterine horns subjected to unilateral decidualization\*

	Total protein (mg%)	Alkaline phosphatase (K.A.U./mg tissue)	Acid phosphatase (K.A.U./mg tissue)
1- Control unstressed Non-decidualized uterine horns.	$1.09 \pm 0.23^a$ (5)	$2.04 \pm 0.35^{a,c}$ (5)	$0.77 \pm 0.09$ (5)
Decidualized horns	$3.48 \pm 0.37^a$ (7)	$4.84 \pm 0.54^a$ (7)	$1.13 \pm 0.3$ (7)
2- Stressed rats Non-decidualized uterine horns.	$1.55 \pm 0.28^b$ (4)	$1.0 \pm 0.14^{b,c}$ (4)	$0.45 \pm 0.23^d$ (4)
Decidualized horns	$4.51 \pm 0.64^b$ (6)	$5.8 \pm 0.63^b$ (6)	$1.53 \pm 0.19^d$ (6)

- Values significantly different from each other in the same vertical row: a,b (P/ 0.001) & c (P/ 0.01) & d (P/ 0.005). Numbers in between parenthesis represent the No. of uterine horns.

- Pseudopregnant rats were stressed by immobilization for 4h/Day for 4 days.

\* Unilateral decidualization was induced using non-surgical instillation of olive oil in one uterine horn of spayed rats after proper hormonal sensitization.

Table (3)  
Effect of immobilization stress on serum levels of total protein, alkaline phosphatase and acid phosphatase of pseudopregnant rats

	Total proteins (g%)	Alkaline phosphatase (K.A.U./100 ml)	Acid phosphatase (K.A.U./100 ml)
Control group	$3.6 \pm 0.17$ (6)	$7.06 \pm 1.25$ (6)	$1.28 \pm 0.12$ (6)
Stressed rats	$2.97 \pm 0.1^a$ (5)	$3.92 \pm 1.12^b$ (5)	$0.76 \pm 0.18^c$ (5)

- Values are means  $\pm$  s.e. and are significantly different from control values at: a (P/ 0.005), b (P/ 0.05) and c (P/ 0.02).



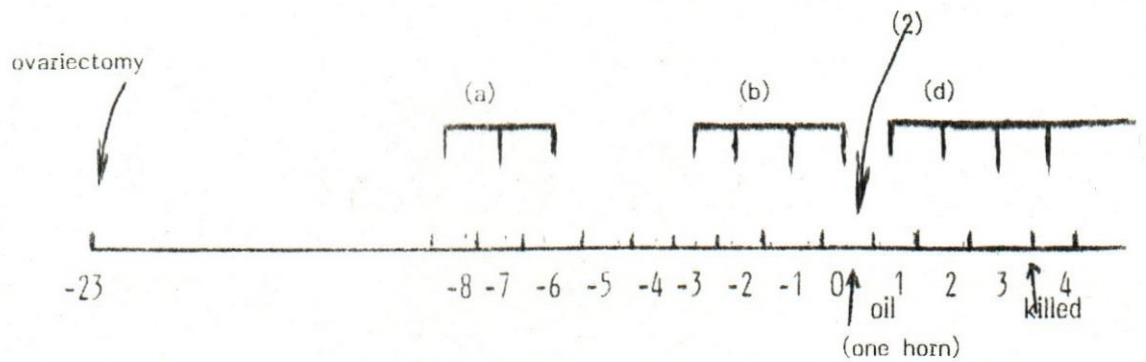


Fig. (1): Hormonal treatment of spayed rats to induce artificial decidualization. a) 0.5ug 17 $\beta$ -estradiol, b) 5mg progesterone, c) 0.1ug estradiol-17 $\beta$ , d) 3mg progesterone plus 200ng oestradiol/Day.



Fig. (2): T.S. of non decidualized horn of control unstressed rat. X 68.

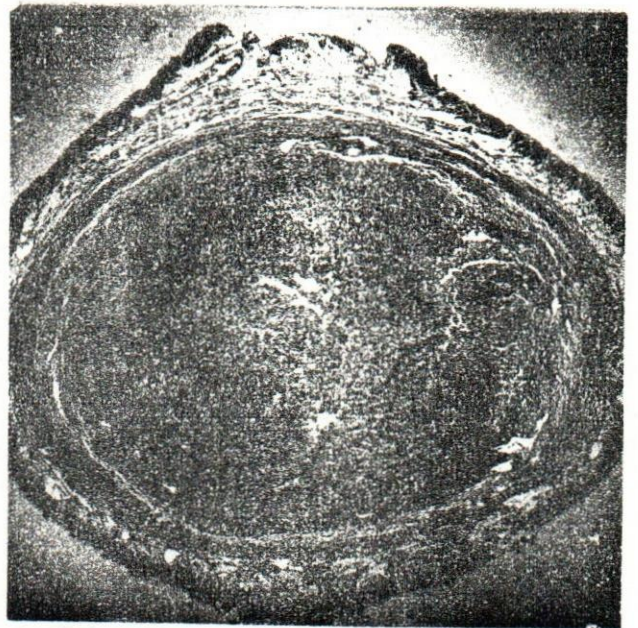


Fig. (3): T.S. of uterus of control unstressed rat on 4th Day of pseudopregnancy after LU instillation of oil, showing distinct DCR, absence of lumen and lamina epithelialis. X 68.



Fig. (4): T.S. of a non-decidualized uterus of a stressed rat, showing reduced diameter. X 68.

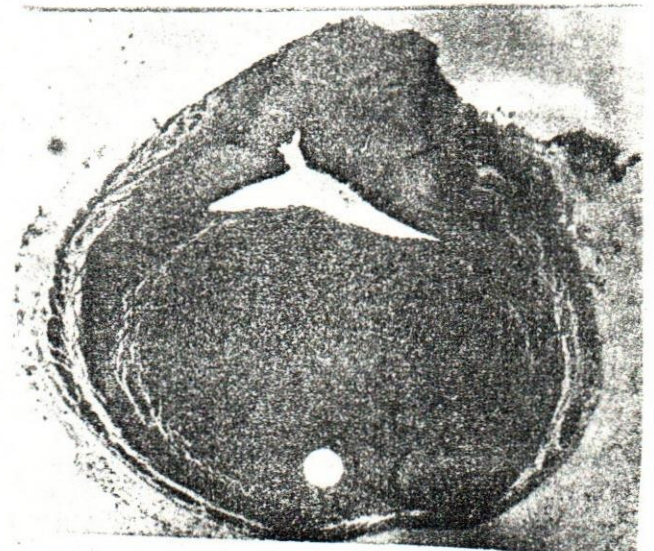


Fig. (5): T.S. of a decidualized uteri horn of a stressed rat on the 4th Day of pseudopregnancy, showing patent lumen, presence of lamina epi., less extensive DCR. X 68.