EFFECTS OF HEAT—STRESS IN RATS*

II. FACTOR(S) RESPONSIBLE FOR REDUCED EMBROYNIC AND/OR FOETAL SURVIVAL PERCENTAGE

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Summary: High ambient temperature (34.4 C° and 60-65% relative humidity (RH failed to maintain optimum embryonic and/or foetal survival rate in ovariectomized pregnant rats given exogenous progesterone and oestrone injections. A still higher ambient environment (36.6 C° and 70-75 % RH) did not affect the decidual cell response (DCR) in pseudo-pregnant rats. Heat stress did not result either in insufficiency or lack of progesterone and hence the latter two are not factors for the heat stress causing embryonic and/or foetal loss.

The possibility of the failure of oestrone being converted to oestradiol or an intrinsic interference of RNA synthesis to be the factors responsible for embryonic and/or foetal loss caused by heat stress has been examined.

Key words: heat stress ovariectomized rats pseudo-pregnant rats decidual cell response embryonic and/or foetal loss oestrone convertion RNA sysnthesis interference

INTRODUCTION

Several reports that high ambient temperatures affect embryonic and/or foetal survival in different laboratory animals are available (1,3,4). However, the basic cause(s) or factor(s) responsible for such losses are not clear and often confused and conflicting. In mice, progesterone insufficiency as the reason for increased embryonic loss during heat-stress was reported by some (1), while others (4) felt that heat stress had a direct effect on the developing mouse embryo.

It was desired, therefore to undertake this study in rats to elucidate a more clearer concept regarding the factor(s) responsible for the high ambient temperature altering either embryonic and/or foetal survival rate. Whether it is progesterone insufficiency or lack of this hormone or any other factor that alters the percentage of embryonic and/or foetal survival, was investigated.

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MATERIALS AND METHODS

Experiment-I to find out whether the gonadal hormone (progesterone) insufficiency is the reason for reduced embryonic and/or foetal survival consequent to thermal stress was studied in 134 pregnant ovariectomized rats maintained with exogenous progesterone and oestrone. The animals procured, maintained and bred for this purpose were similar to those described earlier (6). The ambient temperature and RH for the Control and the Experimental groups were also the same as maintained under trial-1 (6).

All rats were ovariectomized on the afternoon of day-4 (day-0 = day of successful breeding) by bilateral dorso-lateral abdominal incisions under ether anesthesia. A single ligature was placed below the ovary prior to its removal. Gestation was maintained in these rats by twice daily (12±2 hrs interval) injections of progesterone and oestrone according to the dosages in Table I. A pilot study was conducted to determine the optimum ratio of the two steriods for maintenance of pregnancy. Crystalline progesterone* and Estrone** were dissolved in corn oil and injected subcutaneously from the day of ovariectomy until the animals were killed. The volume of injections did not exceed 0.2 *ml.* Heat stress was from day-8 to day-18.

TABLE I: Experimental groups and number of ovariectomized rats in experiment I.

No. of rats in control temperature groups[a]	*Progesterone (mg): Estrone (mcg)*[b]	*No. of rats in heat-stressed groups*[c]
10	3:1	10
23	4:1	23
12	5:1	12
12	6:1	12
10	6:2	10

[a]All control rats maintained at 21.1 C° and 60 to 65% RH from day-8 to day-18.

[b]Ratios shown represent the amount of hormones given daily (in two injections) after ovariectomy on day-4 till day-18 in both control and heat stressed groups.

[c]All heat stressed rats kept at 34.4 C° and 60 to 65% RH from day-8 to day-18.

All rats were lapartomized on day-8. Implantation sites were counted and non-pregnant rats were discarded. Animals were killed on day-18 and live foetuses (having a heart beat), placentae and maternal adrenals and pituitary glands were collected and weighed.

Experiment-II was conducted in 45 normal rats procured and maintained as described under trial - 2 (6). This was to test whether the high ambient temperature prevents uterine decidual cell response (DCR) during pseudo-pregnancy indicating thereby inhibition of progesterone production secretion.

*Crystalline Progesterone was a gift from the Upjohn Co., Kalamazoo, Michigan.

**General Biochemical, Chagim Falls, Ohis.

Pseudo-pregnancy was induced either by fertile mating or by mechanical stimulation of the cervix on the day(s) of vaginal cornification. The ampullary region of the oviduct was surgically removed under light ether anesthesia. Both the procedures were done before 9.00 AM on the day of mating or vaginal cornification. In unmated oestrus rats cervical stimulation was accomplished with a metal rod attached to an electric toothbrush. The rod was pushed through vagina and held against the cervix. Two 15 seconds vibration periods were applied to the cervix. Examination of the vaginal lavages was continued through the experimental period and any rats not maintaining dioestrus lavages until day-4 of pseudo-pregnancy were discarded. The last day of vaginal cornification obtained after mating or cervical stimulation was designated as day-0 pseudo-pregnancy.

Both uterine horns were mechanically trumatized in all rats at 2-00 to 4-00 PM of day-4. Uterine trumatization was done by exposing the uterus through a midventral incision under ether anesthesia and then inserting a hooked needle into the lumen of the uterus at cervical end, passing it to the tubo-uterine junction, and while withdrawing by pressing it against the anti-mesometrial endometrium. All rats were sacrificed on the afternoon of day-9. The uteri were removed, dissected from adnexal tissue and weighed. The weights of adrenal and pituitary glands were recorded. The duration of heat stress and randomly assigned experimental groups are as in Table II.

TABLE II: Experimental groups for measuring the effect of heat on DCR in pseudopregnant rats in experiment II.

Environment	*Day of heat stress*[a]	*No. of rats*
21.1 C°, 60 to 65% RH	Control	15
36.6 C°, 70 to 75% RH	0	15
36.6 C°, 70 to 75% RH	1	15

[a]Day-0 = last day of vaginal cornification after stimulation for pseudopregnancy.

Results were analysed statistically using student 't' test for unequal subclass number.

RESULTS AND DISCUSSION

Evidence of heat stress in ovariectomized and pseudo-pregnant rats were evident by an increase in the rectal temperature from 37.2 C° to 40.5C° with maximum reading observed in 2 hrs since exposure to the high ambient temperature.

In experiment-I, foetal mortality was significantly increased in ovariectomized pregnant rats, given various levels of progesterone and oestrone, on exposure to 34.4 C° and 60-65 % RH from day-8 to day-18, when compared with controls kept at 21.0 C° and 60-65 % RH. The percentages of foetal survival of the different levels of hormones administered is shown in Table III.

TABLE III: Percent foetal survival in heat stressed ovariectomized rats given various levels of progesterone and estrone [a].

Daily progesterone (mg): Estrone (mcg)	*Temperature and relative humidity from day-8 to day-18*		*% Decrease in survival due to stress*
	21.1 C°, 60 to 65% RH	34.4 C° 60 to 65% RH	
3:1	74.4 ± 9.5 (10)[b]	41.8 ± 3.9 (10)	—32.6
4:1	65.3 ± 6.0 (23)	58.6 ± 6.3 (23)	— 6.7
5:1	46.2 ± 10.2 (12)	38.5 ± 12.4 (12)	— 7.7
6:1	47.8 ± 9.9 (12)	23.6 ± 6.3 (12)	—24.2
6:2	26.1 ± 7.3 (10)	5.0 ± 3.0 (10)	—20.9
All above groups	51.9 ± 8.7 (67)	33.5 ± 7.6 (67)*	—18.4

[a] All ovariectomies were performed on day-4; rats were then injected with progesterone and estrone twice daily until killed at day-18. The number of implantation sites were counted by laparotomy on day-8 (day-0 = day of mating).

[b] Values are percent survival ± SE with the number of rats in parentheses. Foetal survival for the group is calculated by averaging the percent of foetuses surviving from day-8 to day-18 in each rat in the group.

*Different than rats kept at 21.1 C° ($P < .05$).

Increasing the levels of progesterone or progesterone and oestrone therapy in the animals caused further loss, more so in heat-stressed animals than those kept at control conditions. Heat stress apparently does not increase the requirements of gonadal hormones during pregnancy in this study. It is, therefore, inferred that the high ambient temperature might interfere with the utilization of these hormones. Our results confirm the findings of Bolt and Spies (3) who also felt that one of the factors may be that heat stress in some way prevents the convertion of oestrone to oestradiol (the more active form of eostrogen). This stagnation could be a factor in reducing foetal survival in this study also. Another possibility is that twice daily injections of the hormones do not maintain a uniform circulating level of gonadal hormones and during low periods the heat stress induces mortality in the foetuses.

In experiment-II, pseudo-pregnant rats were heat stressed at 36.6 C° and 70-75% RH and the uterus was subsequently traumatized to induce decidual cell response. Treatment on day-0 or day-1 did not affect the uterine growth (Table IV) compared with controls (21.1 C° and 60-65 % RH). Heat stress as given in this experiment did not affect gonadal hormone secretion sufficiently to prevent decidual cell response or prevent the uterus from responding to these hormones. These lend support to the thesis that thermal stresses are not endocrine mediated. Similar view was also reported by Tompkins (8).

Krishnan and Daniel (5) had reported that a protein called 'Blastokinin' in uteri of rabbits was required for continuous development of foetuses (5). Heat affecting the production of

this protein and hence embryo development has also been reported (4). While this may not be the reason for foetal losses observed between day-8 and day-18, there is the possibility of the elevated temperature intrinsically preventing the RNA synthesis as suggested by Elliot *et al.* (4).

TABLE IV: Decidual cell response in rats heat stressed (36.6 C°, 70 to 75% RH), on day-0 or day-1 of pseudopregnancy[a].

Day of heat stress[b]	*Number of rats*	*Av. uterine wt.*$\pm$*SE* (*mg*)[c]
Control	15	4320 $\pm$ 379
0	15	3950 $\pm$ 387
1	15	4340 $\pm$ 137

[a] Day-0 is the last day of vaginal cornification after the stimulus for induction of pseudopregnancy.

[b] Control rats were kept at 21.1 C° and 60 to 65% RH. Treated rats were returned to control temperature after 24 hrs heat stress.

[c] Both uterine horns of each rat were mechanically traumatized at mid-day of day-4 and were collected and weighed on day-9.

In preliminary studies ova collected from the oviducts of three bred rats on day-0 when observed under a microscope revealed all the ova in one cell stage and had sperm in the zona pellicuda. But in two rats on examining on day-2 the ova were in two cell stage. Earlier Narendranath and Kiracofe (6) reported that day-0 is critical for the heat stress in rats, causing losses and also the presence of a delayed effect of heat on mortality of the conceptors.

Hence, it is suggested now, that though thermal stress failed to reduce the decidual cell response in rats, it acts directly on the developing conceptors during first clevage, perhaps denaturing some component such as RNA.

It is, therefore, concluded that during high ambient temperature, there is neither lack nor insufficiency of progesterone requirements for the developing embroys/foetuses. The results obtained suggest either inhibition of oestrone being converted to a more active oestrogen (oestradiol) during thermal stress or the latter might act directly on the ova during the first clevage in some intrinsic way upsetting RNA synthesis.

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