ENDOCRINE-CLIMATIC RELATIONSHIPS THAT INFLUENCE REPRODUCTIVE TRAITS IN EWES AND RATS

by

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INTRODUCTION

It is economically important for the female of domesticated species to possess ability to exhibit estrus, be bred, ovulate a desirable number of fertilizable ova, provide a proper environment for normal embryonic development and to successfully give birth to young. The influence exerted by environment upon these factors is not fully understood, but lowered fecundity is well recognized in farm animals, especially sheep, living in unfavorable environments. Light, relative humidity, barometric pressure, season, and ambient temperature are factors known to influence the desirablity of an environment.

This study was designed to analyze only one of these environmental elements, namely high ambient temperature. The mechanism by which high ambient temperature exerts a detrimental influence on embryo survival in the female rat and ewe was sought in hope that knowledge gained might contribute to the final solution of the problem of poor reproductive performance in farm animals living in hot climates.

Two hypotheses were tested: (1) high environmental temperature lowers embryo survival either via reduced or altered ovarian steroidogenesis; (2) high environmental temperature influences the effectiveness of a given level of ovarian hormones at the target organ.

The first hypothesis was tested in ovariectomized and intact pregnant ewes and rats exposed to high environmental temperature with embryo survival as the end-point. If heat had greater detrimental effect on embryo survival in the intact female than in the ovariectomized female, there would be suggestive evidence that ovarian steroid production was altered.

The second hypothesis was tested using the Astwood 6-hour biological estrogen assay. The assay animals were subjected to either a high or moderate experimental temperature and the uterine response to 6 standard doses of either estrone or estradiol-17B was compared at each temperature.

The latter experiment was attempted because many of the heat sensitive events of reproduction are also estrogen dependent.

LITERATURE REVIEW

Ewes must be bred during the hot summer months if spring market lambs are desired. This practice usually results in a long lambing season and low lambing percentage due to difficulty in settling ewes early in the breeding season. Hulet et al. (1956) noted fertility of dark-faced ewes was lowest in the early breeding season with 64.3% (27 of 42) of the ewes failing to conceive before September 16 as compared to 34.3% (12 of 35) from September 16 to October 25. Dutt (1954) and Hulet et al. (1956) flushed reproductive tracts of ewes bred early in the breeding season and classified a high percentage of the ova recovered as abnormal. Dutt (1954) also noted that a high percentage of the ova recovered were not fertilized and concluded this was the most important single factor contributing to early season infertility. The importance of a fertile potent male as well as fertile females is obvious.

Elevated ambient temperature is known to effect both the fertilizing ability of the ram (Moore et al., 1924; Moore, 1924; Steinberger et al., 1959; Dutt, 1960; MacFarland et al., 1959; Phillips et al., 1934; El-Sheikh and Casida, 1954; and Dutt et al., 1957) and ewe (Dutt, 1960). Alliston et al., (1961) housed rams and ewes in controlled environments of 65° and 92° and concluded differences in fertilization rates are more dependent on factors

involving the male than the female when the treatment period includes the time of mating. High ambient temperature may have its effect in the male directly on sperm fertilizability and/or indirectly by changing the environment of the reproductive tract of the ewe thereby altering the sperm cell in utero prior to ovum penetration. Alliston and coworkers further speculated that in ewes receiving heat treatment shortly after fertilization, the loss in potential young occurs by cessation of embryo development during early cleavage possibly due to a direct effect on the ovum. One should also point out implantation as an alternate site for the effect of high ambient temperature.

In addition to reduced fertilization rates, Yeates (1953) reported that high temperature reduced birth weight of lambs carried by ewes exposed to elevated temperature during pregnancy. Ewes exposed to 107°F for 7 hours daily during the first 1/3 of gestation, last 2/3 or the entire gestation period gave birth to lambs averaging 7 lbs. 13 oz., 4 lbs. 1 oz., and 1 lb. 2 oz. respectively, compared with 9 lbs. 5 oz. for control ewes. The interrelation of temperature and nutrition was also tested by Yeates (1953) in three groups of ewes. Lambs from ewes receiving either a high or a low plane of nutrition had birth weights 8 lbs. 6 oz. and 6 lbs. 13 oz., respectively, while a group of ewes on a low plane of nutrition plus 102°F for 7 hours daily throughout gestation had lambs weighing only 4 lbs. at birth. Yeates suggested that the stress of the high temperature resulted in a reduction in blood supply to the gravid uterus which caused the harmful effect on fetal development. He further speculated that blood and nutrients may have been diverted from the uterus in an effort to compensate for the low supply of energy available to the dam for body maintenance. Since high

temperature stress in addition to the low plane of nutrition did not reduce fetal organ weight below that of the low plane of nutrition group, Yeates speculated that the detrimental effect of the two treatments on birth weight may be via different mechanisms. Plane of nutrition has also been shown to reduce the testosterone output of the testes of rams as reported by Setchell et al. (1965).

Shearing was reported by Dutt et al. (1959) to improve heat tolerance in ewes exposed to 90°F and 60% relative humidity (RH). Treatment was initiated 12 days after last estrus and ended either 3 days or 25 days after breeding. Control ewes, housed in an open-sided barn, had 92% cleaved ova compared with 64% for sheared-hot and 40% for unsheared-hot ewes. The unsheared-hot group also had more ova classified abnormal than control ewes and in addition, had only a 9% lambing rate. Dutt theorized that ova are most sensitive to high temperature while still in the oviduct. Dutt (1963) tested this theory by exposing ewes to an artificial environment of 90°F and 60-65% RH beginning on different days of the cycle and continuing until 24 days after mating. The temperature-treated ewes were then returned to the outside environment to run with control ewes. Lambing percentages were 80 and 90 for the two years in control groups, 0.0% when exposed days 0-24, 20% both years for the 1-24 days treatment, 40 and 30% for the days 3-24 group, and 40 and 50% for the group exposed days 5-24. Again evidence points to a critical period for exposure to high temperatures from mating till approximately 8 days after mating.

With the knowledge that the ewe is most susceptible to high temperature stress while the ova is in the oviduct, Alliston and Ulberg (1960) attempted to determine if the ova were affected directly or if the oviducal and/or uterine environment was rendered unfavorable by the elevated temperatures.

These workers transferred embryos 60-84 hours after mating from donor ewes maintained at 70° or 90°F into recipient ewes maintained at 70 or 90°F. They found 18 of 34 ewes pregnant at laparotomy 25-30 days after mating in the 70° to 70° group, 12 of 38 in 70° to 90°, and 2 of 30 pregnant in the 90° to 70° group. In a similar experiment Alliston and Ulberg (1961) reported 56.5% survival (13 of 23) in the 70° to 70° group, 24% (6 of 25) in the 70° to 90° group and 9.5% (2 of 21) in the 90° to 70°F group. Higher survival percentage in the 70° to 90° than the 90° to 70° group suggest that the detrimental effect of high temperature was realized more by the donor than the recipient ewes.

Shah (1956) transferred rabbit embryos from does maintained at 95.9°F to does maintained at room temperature on day 6 of pregnancy and noted no difference in survival percentage from that observed when transfer was made between two control temperature groups. Shah concluded that no detrimental effect had been realized by the ova prior to day 6 and any detrimental effect thereafter must be related to an unfavorable uterine environment. This is in contrast with results reported by Chih-Yun (1948) for the rat, who concluded that the pronuclei and early cleavage state of embryonic development is the stage most susceptible to hot environments. Fernandz-Cano (1958a, 1958b) found the rat very resistant to high temperature after day 8 of gestation which is again in agreement with results reported for the ewe. The presence of tertiary membrances on the rabbit ova may contribute to the apparent species difference.

MacFarland et al. (1957) found that rats living at 35°C lost up to 50% of their embryos compared with 7% resorption in the control group maintained at 22-28°C. Injections of progesterone from day 5 to day 20 of gestation

decreased the rate of resorption. Thyroxine, vitamine supplements and high protein diet also tended to improve embryo survival in heat stressed rats. Acclimatization for 2-10 weeks at 35° before mating significantly reduced fetal loss but the number of corpora lutea formed was also reduced. Estrus was not inhibited in this trial as was reported by Chang et al. (1959) in a similar experiment. Injection of cortisone increased the rate of resorption in both the heated and unheated groups.

Aldred et al. (1961) subjected mice to the stress of 104°F for 5 hours on days 1 and 2 and noted increased embryo resorption at autopsy on day 17 (14% in controls vs. 32.5% in heat treated). Progesterone (1 mg/kg body weight) on days 1 and 2 reduced embryo loss in the heated group to 7.0%, which was less than that obtained in the unheated control group, i.e., 14%. This apparent improvement in embryo survival with progesterone therapy is in agreement with results reported by MacFarland et al. (1957) and suggests that progesterone is not available in sufficient quantities for proper embryo survival during high temperature stress. It would be of interest to determine if the rate of progesterone synthesis is reduced by high temperature stress or if the maternal demand for progesterone is merely increased.

Ogle (1934) found mice to be more resistant to continual 90°F temperature than when the environment was changed from 64° to 90° every 12 hours. Average litter size was 5.6 in 20 females in continual hot environment and 2.3 for 3 of 21 females which became pregnant in the changing temperature group.

Chih-Yun (1948) exposed pregnant rats to high ambient temperatures for 1 or 4 hours starting at various times after insemination and found that the

embryos do not die immediately after treatment but usually live until day 5-7 of gestation which is approximately 1-4 days after expected implantation. Austin (1956b) supported these findings with the observation that heat treatment (39°C 1-4 hours shortly after insemination) accelerates embryonic development while cold environmental temperatures tend to retard embryo development before implantation. Chih-Yun (1948) postulated that the uterine development prior to implantation procedes at a rate optimal for embryo development in normal environments, and changes little under either hot or cold environmental influences. He also postulated that the embryo alters its rate of development in response to the environmental stimuli present either directly or indirectly via uterine secretions and in the case of high temperature, use up its stored food supply before the uterus is properly prepared to supplement the embryo. High temperature might cause sub-optimal preparation of the uterine endometrium and thereby discourage implantation. One might speculate that improper level or balance of ovarian hormones may result in impaired uterine function due to altered corpora lutea function.

Denison and Zarrow (1955) report that the rat has lengthened estrual cycles while living in a cold environment. The proestrous and estrous phases are lengthened which suggests an increase in the level of FSH. Thyroxine (50 mg/day) returned the cycle to normal. Ogle (1934) found a delay in vaginal plate opening in mice living in hot environments. Chang et al. (1959) noted extended estrual cycles with both high and low temperatures as well as low atmospheric pressure. He also noted an increase in the number of corpora lutea formed in the heat treated group and attributed the differences in corpora lutea numbers and extended cycles to an increase in ACTH secretion and a reduction in gonadotropin secretion. The increase in number of

corpora lutea is not in agreement with results of Austin (1956b) who showed a reduction in number of ova ovulated following exposure to high temperature.

Cold temperature stress (14-18°C for 10 hours) and hypoxia resulted in no detrimental effect when treated before day 13 of gestation in the rat, but after this date resorption of young, increased instance of still births, decreased birth weight and delayed onset of parturition occurred (Giaja, 1940). Since hot environments are detrimental to embryo survival early in gestation and cold environments are harmful to the latter part of pregnancy, a different mechanism of action for the two environments is suggested.

During a stressful experiment, the test animal may experience a depression in appetite which causes a reduction in feed intake. The limited food intake in itself may result in a detrimental effect which can be erroneously attributed solely to the stressor being studied. McClure (1959, 1961a) noted embryonic mortality in mice fasted for 48 hours. When females were fasted for two 48 hour periods prior to implantation, no embryo survival was noted. Adrenalectomy did not decrease early embryo mortality in the fasted mice which suggest that the adrenal is not involved in the effect noted by McClure. Progesterone or human choronic gonadotropin (HCG) produced limited improvement in embryo survival (McClure, 1961b).

Velardo (1957a) showed ACTH injected into pregnant rats reduced litter size and produced large numbers of still births. The most detrimental treatment was begun on the day of mating. This treatment had no effect in adrenalectomized rats (Velardo, 1957a), and was not mediated via the ovary (Burdick et al., 1941).

Mills (1960) states that following release from the adrenal, corticoids are transported via the blood to distant sites in a bound form. Even though

these steroids are usually bound to transcortin during transportation, the form active at the tissue level is the non-bound molecule. Mills (1960) noted that an increase in body temperature resulted in a decrease in unbound cortisol. Estrogen injections caused an increase in the bound form. From these observations, one might speculate that high environmental temperature could result in elevated corticoid activity even though no actual increase in amount of adrenal secretion occurred. Either an increase in body temperature and/or decreased estrogen level could potentiate the detrimental effect of the corticoids on embryo survival.

Although the placenta is thought to be capable of maintaining pregnancy in some species late in gestation, the secretions of the ovary are essential through the period of gestation when the detrimental effect of high temperature stress is most evident in the rat and ewe. Following ovariectomy on day 13 of gestation in the rat, Zeiner (1943) found that the average continuation of pregnancy was only 80 hours without replacement therapy. If the operation was performed on day 9, resorption occurred within 24 hours (Frazer, 1954). Progesterone treatment allows pregnancy to be maintained if rats are ovariectomized after implantation (Yochim and Zarrow, 1961; Lyons, 1943; Lerner et al., 1962 and Hall, 1957), but addition of small amounts of estrogen are necessary for proper nidation (Yochim and Zarrow, 1961; Lyons, 1943, and Learner et al., 1962 and Cochrane et al., 1957). Lyons (1943) used 4 mg progesterone and 1 ug estrone daily to maintain pregnancy in rats. Foote et al. (1957) successfully maintained pregnancy in 14 of 21 ovariectomized ewes with 1 mg progesterone and .25 ug estrone per 5 lb. body weight. Frazer (1954) postulated that the lack of ovarian hormones causes embryonic death because the uterine myometrium is not capable of stretching to accommodate the growing embryo.

Boving (1959) has discussed the general endocrine influences on implantation as has Shelesnyak et al. (1963). Progesterone is necessary for proper decidualization, but estrogen and/or histamine is involved in the mechanism which initiates implantation (Shelesnyak et al., 1963; Boving, 1959 and Johnson et al., 1958). If a stressful stimulus could sufficiently reduce the effectiveness of either of these two hormonal mechanisms, implantation would be influenced.

Traumatization of the uterine horn of pseudopregnant or ovariectomized progesterone treated rats results in formation of a deciduomata. This response is completely inhibited when rats are given pregnanediol, testosterone, 11-desoxycorticosterone, corticone, or ACTH (Hisaw and Velardo, 1951). Several anti-estrogens and estrogens are also reported to prevent deciduomata formation (Emmens et al., 1962). They noted that local injections produced local inhibition. If any of these compounds which tend to inhibit decidualization were present in excess, implantation might be interfered with.

Estrogen has been linked to several critical events concurrent with the period in gestation when high environmental temperatures are most detrimental to embryo survival. The effect of high temperature stress may therefore be linked to low estrogen levels. Such connection would suggest that high environmental temperatures alter the production or effectiveness of endogenous estrogens.

A quantitative 6 hour biological assay for estrogen was devised by Astwood in 1938. The assay measures the increase in wet weight of the uterus of the immature rat 6 hours after the estrogen injection. Later workers have found that extracellular water increases for about 6 hours postestrogen injection and then declines slightly only to rise to a peak by 42 hours and

returns to normal by 66 hours after injection (Talbot et al., 1940 and Zuckerman et al., 1949). The second peak in weight increase is due to both extracellular water and to actual growth as evidenced by an increase in protein, ribonucleic acid and phospholipid synthesis (Nicolette and Gorski, 1964), increase in cell division as shown by colchicine pretreatment (Spaziani, 1963) and increase in electrolytes and plasma proteins (Spaziani et al., 1958).

Spaziani et al., (1958) indicates that the early accumulation of water, electrolytes and plasma proteins following estrogenic stimulation of the uterus are secondary manifestations of the hyperemia and increased capillary permeability resulting from estrogen-induced release of endogenous histamine. These workers were able to show a significant depletion in uterine histamine concentration within 4 hours after estradiol 17-B injection. Solutions of histamine or synthetic histamine releasing agents injected intraluminally produced results comparable to those resulting from estrogen injection (Spaziani et al., 1959b). Spaziani (1963) suggests that the cellular action of histamine results in an increase in capillary permeability and increased vasodilation.

Velardo (1958) found that maximal uterine growth, in a 72 hour assay, was obtained with a daily injection (s.c.) of 1.0 ug 17-B estradiol, 5.0 ug estrone, 50.0 ug 16 β hydroxyestrone, 50.0 ug 16 B hydroxyestrone or 20.0 ug 16 - epiestriol. He used ovariectomized, adrenalectomized rats as test animals. Hisaw et al., (1954) noted that estradiol-17 B was approximately 10 times more effective than estrone. He found that 1.0 ug estradiol-17 B and 10.0 ug estrone produced a comparable increase in uterine weights at the end of a 72 hour test. Astwood (1938) reported that estradiol-17 B was 12 times more effective than the same quantity of estrone with 0.1 ug estradiol-17 B and 1.0 ug estrone producing maximal uterine weight increase.

Studies have shown that the effectiveness of an injected estrogen can be greatly altered by giving various other steroids along with the test estrogen. Some of these steroids include estriol and 16 epiestriol (Velardo, 1958; Hisaw et al., 1954; Edgren, 1961; Dorfman et al., 1961a; Dorfman, et al., 1961b and Edgren et al., 1961), testosterone (Robson, 1950) and progesterone (Robson, 1950). Considerable evidence indicates cortisol and other gluco-corticoids suppress the response of uterine hyperemia and water inhibition produced by estrogen injection (Spaziani, 1963; Spaziani, et al., 1959a, 1959b; Szego, 1948 and Nicolette and Gorski, 1964). Spaziani and Szego (1958) and Spaziani et al., (1959a, 1959b) have concluded that the inhibition of the estrogen response produced by cortisol is due to a non-specific decrease in capillary permeability in the uterus.

From this brief discussion it can be seen that hyperthermia could have a depressing effect on the response of the Astwood assay in several ways. The effect could be directly due to elevated body temperature, indirectly involving the adrenal or other organs or via an alteration in estrogen metabolism.

The uterus of the mouse incorporates tritiated estrone and estradiol when given subcutaneously (Stone, 1963) or intravenously (Stone et al., 1963). When the uterus of the estradiol treated mouse was extracted and chromatographed, the radioactivity was almost entirely ether soluble and moved with the standard estradiol fraction. The uterine extract of the estrone treated mouse moved with both estrone and estradiol. These results suggest that the injected estrone was converted to estradiol. The injected estradiol was evidently not converted to estrone, at least no labeled estrone could be recovered from the uterus of the treated mice.

Jensen and Jacobson (1962) found the uterus of the rat incapable of incorporating and retaining estrone for prolonged periods in contrast to a slow rate of disappearance of estradiol. Following injection of tritium labeled estrone, primary labeled estradiol and a little estrone was recovered from the rat uterus. When labeled estradiol was given, the only radioactive substance found in the uterus was free estradiol. The amount of labeled estradiol recovered following injection of labeled estrone was about 1/10 the amount found after injecting the same quantity of labeled estradiol. Jensen and Jacobson (1962) concluded that estradiol stimulates uterine growth without undergoing metabolic transformation, but estrone exerts its hormonal action upon the uterus after first being converted to estradiol (the active form). The possibility that estrone is first converted to estradiol before any estrogenic response is observed on the uterus could explain the 10:1 ratio of effectiveness of estradiol and estrone when compared on the basis of increased uterine weight, maintenance of pregnancy and decidua formation.

If, in some manner, hyperthermia decreases the rate of conversion of estrone to estradiol and estradiol is the estrogen active at the uterine level, high temperatures should also cause a reduction in response to the Astwood estrogen assay. If so, high temperature might also impair implantation or the maintenance of pregnancy mechanisms which are dependent upon estrogen.

EXPERIMENT 1

Methods and Materials

Forty adult female Sprague-Dawley rats were housed under a 14 hour light-10 hour dark regime and provided with Purina lab chow and water

ad libitum throughout the experiment. Females were housed with fertile males and examined each morning for the presence of a vaginal plug or sperm in the vaginal smear. Upon finding evidence of copulation (day 1) rats were randomly assigned to one of the four following groups: Group 1, intact-control environmental temperature (70-80°F and 55-65% relative humidity); Group 2, intact-high environmental temperature (96-98°F and 35-45% relative humidity); Group 3, ovariectomized-control environmental temperature; Group 4, ovariectomized high environmental temperature. Rats assigned to groups 1 and 3 were caged individually. Rats assigned to groups 2 and 4 were caged together and placed in the environmental chamber (96-98°F and 35-45% RH) the afternoon of day 1 through the afternoon of day 8. The ovaries were removed from rats in groups 3 and 4 on the afternoon of day 3 (approximately 60-68 hours after mating). A dorso-lateral approach was used, with ether anesthesia. Care was taken to avoid traumatization of oviduct. Pregnancy was maintained in the ovariectomized rats by daily subcutaneous injections of 4 mg progesterone and 1 ug estrone in 0.2 ml sesame oil. Intact rats were uninjected. Uterine swellings were counted at laparotomy on day 8 in all rats. Embryos and placenta were removed, counted and weighed at day 18. Embryo survival was calculated by comparing uterine swellings counted at day 8 with number of embryos alive at day 18. Corpora lutea were counted at day 3 in the ovariectomized groups or at day 18 in the intact groups.

Statistical differences were computed by analysis of variance using a randomized complete block design with temperature as treatment and ovariectomy as block. Fishers LSD was used to test group differences (Snedecor, 1956).

Results

Pregnant intact and day 3 ovariectomized rats subjected to 96-98°F, 35-45% RH from the afternoon of day 1 (vaginal sperm found a.m.) until day 8 had a smaller number of embryos alive at day 8 and day 18 and a decreased percentage of embryos surviving from day 8 to 18 when compared to similar intact and ovariectomized rats exposed to 70-80°F, 55-65% RH. The differences were not significant, however. There were also no significant treatment X block interactions for the three end points.

Percentage of embryo survival from day 8 to 18 in the ovariectomized groups was not statistically different from that of the intact groups. The number of embryos alive at day 8 was 10.3, 9.3, 10.0 and 9.4 for the intact, control temperature (group 1); intact-high temperature (group 2); ovariectomized control temperature (group 3); and ovariectomized, high temperature (group 4) groups, respectively.

Number of embryos alive at day 18 was 8.9, 6.8, 8.1, and 6.8 for groups 1 through 4, respectively. Percent embryo survival day 8 to 18 was 82.9, 58.6, 81.0 and 69.9% for groups 1 through 4, respectively (Table 1).

Treatment, block and treatment X block effects were all significant ($P < .01$) for number of corpora lutea counted. Corpora lutea counts were 12.4, 15.6, 11.6 and 10.9 for groups 1, 2, 3, and 4, respectively (Table 1). Fisher's LSD showed the number of corpora lutea in the intact, heat group to be significantly higher than number of corpora in the other 3 groups. The comparisons, however, are not completely valid as corpora were counted at different times in the intact and ovariectomized groups.

Eighteen day average embryo weight were increased significantly ($P < .01$) by ovariectomy and progesterone treatment. No treatment X block interaction

Table 1. The effect of high temperature and/or ovariectomy on embryo survival in rats. Corpora lutea were counted at day 3 in ovariectomized groups or at day 18 in intact groups.

Groups	No. of rats	Ave. No. CL	Ave. No. day 8**	Embryos day 18**	Embryo survival(%) day 8-18**
1. (Intact - 70° to 80°F)	9	12.4	10.3	8.9	82.9
2. (Intact - 96° to 98°F)	9	15.6*	9.3	6.8	58.6
3. (Ovariectomized - 70° to 80°F)	10	11.6	10.0	8.1	81.0
4. (Ovariectomized - 96° to 98°F)	10	10.9	9.4	6.8	69.9

*Significantly larger ($P < .05$) than other groups

**Values not significantly different ($P < .05$)

Table 2. The effect of high temperature and/or ovariectomy on the weight of embryonic tissue at day 18 of gestation.

Groups	No. of rats	Ave. embryo weight(mg)**	Ave. per litter placental membrane weight(mg)*
1. (Intact - 70° to 80°F)	9	843.4	401.3
2. (Intact - 96° to 98°F)	7	692.0	399.9
3. (Ovariectomized - 70° to 80°F)	10	1,121.0	688.8
4. (Ovariectomized - 96° to 98°F)	10	943.3	816.7

*The ovariectomized groups were significantly ($P < .05$) heavier than intact groups. No other significant differences.

**The ovariectomized - 70° to 80°F group significantly ($P < .05$) heavier than the intact 96° to 98°F group.

was observed. Group means were 843.4, 692.0, 1,121.0 and 943.3 mg for groups 1, 2, 3, and 4, respectively (Table 2).

Average placental weights per litter of the two ovariectomized groups were significantly larger than intact groups ($P < .01$). Values for the intact control and the intact heat groups were 401.3 and 399.9 mg, respectively. Values for the control and heat-treated ovariectomized groups were 688.88 and 816.7 mg, respectively (Table 2).

Discussion

Exposure to 96-98°F from the afternoon following mating (day 1) until day 8 had no significant effect on number of embryos alive at day 8 or 18, percent embryo survival from day 8 to 18 or weight of placental membranes. These results are in contrast with results reported by Chih-Yun (1948); MacFarland et al., (1957); Fernandez-Cano (1958a, 1958b) and Chang et al. (1959). This discrepancy may in part be due to the use of a low relative humidity in the high temperature treatment and the high relative humidity used in the control temperature of this experiment compared with that used by other workers. The rate of embryo survival in the intact-control temperature group (70.9% day 0 to 18) is considerably lower than the 93% reported for this temperature by MacFarland et al., (1957). The loss of nearly 20% of the potential embryos in the control group suggests that embryo survival in this group was not optimal.

Since no significant reduction in embryo survival was realized between the intact-high temperature group and the intact-control group, one could not test the original hypothesis (does high temperature stress reduce ovarian steroid production or does thermal stress alter the utilization of these hormones?).

The number of corpora lutea was significantly ($P < .01$) affected by both temperature and ovariectomy but there was a significant treatment by block interaction. The treatment and block effects are confounded with the difference in time corpora were counted in the intact (day 18) and the ovariectomized group (day 3). The difference between the 12.4 average corpora lutea counted in the intact-control group, and the 15.6 average number counted in the intact-heat treated group, is probably not due to more ova being ovulated in the heat treated group as reported by Chang et al. (1959), because heat treatment was not initiated until after rats had ovulated (Everett et al., 1949). High ambient temperature may have either resulted in accessory ovulations or possibly the corpora of the pre-treatment cycle may have re-established functionality. In either case corpora present at day 18 would not necessarily represent the number of ova ovulated. The comparisons of embryo survival are therefore limited to actual number of embryos present rather than values based on corpora lutea counts.

The significant increase ($P < .05$) in average embryo weights and average placental membrane weights observed in ovariectomized groups compared to intact groups are possibly related to an improved ability of the uterus to furnish nutrients to the developing embryonic tissue. Kao et al. (1961) reported there is about twice as much blood in the rabbit uterus under progesterone domination than in estrogen dominant controls. Possibly the combination of exogenous estrogen and progesterone was such that heavier than normal placental membranes were formed which resulted in improved nourishment to the embryos and thus the heavier embryos in the ovariectomized groups. Yochim et al. (1961) also reported heavier embryos in ovariectomized rats with several doses of estrogen and progesterone. It is also possible that exogenous estrogen and/or progesterone hastened implantation and the

heavier embryonic tissue in the ovariectomized groups was due to a longer postimplantation period of development. Canivenc et al. (1953) reported progesterone injected locally in the uterine wall caused early implantation in lactating rats. Although estrogen is recognized (Shelesnyak, 1957) as being necessary for implantation in the rat, Cochran et al. (1957) failed to obtain precocious implantation with exogenous estrogen in ovariectomized rats.

Reduced mean embryo weight was noted at day 18 in the high temperature groups ($P < .05$). Temperature, however, caused no significant effect on mean placental weights. No explanation for these differences is obvious.

Pregnancy was successfully maintained in rats ovariectomized on day 3 with a daily subcutaneous injection of 4 mg progesterone and 1 ug estrone (10.3 vs. 10.0 average number of embryos alive at day 18 in intact-control and ovariectomized-control groups, respectively). Lyons (1943) was less successful with this dosage in hypopysectomized, ovariectomized rats. Yochim et al. (1961) reported several combinations of estradiol and progesterone (including the dosage used in this experiment) capable of maintaining pregnancy at a rate similar to control rats, however, ovariectomy in their experiments were performed on day 12. Even though pregnancy was maintained in ovariectomized rats at a rate comparable with controls, the heavier embryo and placental membrane weights in ovariectomized groups suggest that the dose of estrogen and progesterone was not equivalent to that produced endogenously in the intact pregnant rat. Possibly a different ratio of estrogen and progesterone or a lesser total amount of the hormones given twice or more times daily would more closely approximate the normal ovarian secretions during pregnancy.

EXPERIMENT 2

Methods and Materials

Forty-six, 5 to 7 year old western ewes were used to study the effect of high ambient temperature (90°F, 45-55% RH) on embryo mortality in intact sham operated and ovariectomized ewes. Thus nine experimental groups were used. Group 1: intact, 70°F; Group 2: ovariectomized, 70°F; Group 3: sham operated, 70°F; Group 4: ewes in outside environment during 70° run; Group 5: intact, 90°F; Group 6: Ovariectomized, 90°; Group 7: sham operated, 90°F; Group 8: outside environment during 90° run; Group 9: ewes anesthesized with pentobarbital sodium but unoperated 70°F. Due to the limitations of the environmental chamber, the experiment was conducted in two phases. Groups 1-4 were run through July 31 through August 25, 1963. The second phase of the experiment (Groups 5-8) was conducted September 14 through October 10. An outside control group of ewes was maintained during both phases to help evaluate possible seasonal effects. Group 9 was included after 5 of 6 sham operated ewes were not pregnant at day 22, and was conducted in November.

Ewes with previous cycle history were placed with fertile, raddled rams and observed at 12 hour intervals. During each phase of the experiments marked ewes were then handmated to a different fertile ram and placed at random in their respective group. Ewes were handmated again 12 hours later. Onset of estrus was designated day 0.

Bilateral ovariectomy or sham operation was performed 48 hours post-onset of estrus via a mid-ventral incision under pentobarbital sodium anesthesia. Sham operation consisted of disecting the ovary free from its attachment to the uterus and oviduct. No ligations were made. Ewes

undergoing surgery were removed from the environmental chamber for approximately 4 hours.

Pregnancy was maintained in ovariectomized ewes with daily injections of 20 mg progesterone and 4 ug estrone per 100 lbs. body weight, except the day of surgery and the following day when 1/2 and 3/4 that amount was given. Intact ewes were uninjected. All ewes were slaughtered on day 22, postmating.

Embryos and placental membranes were fixed in Bouin's fluid immediately after tissue had been removed and the presence or absence of a heart beat had been established. Embryos were weighed and crown rump measurements taken after samples from each phase of the experiment had been obtained. Excess Bouin's was removed by blotting on filter paper until no trace of yellow remained on paper.

Group differences in embryo weight, placental membrane weight and crown rump measurements were statistically analyzed by analysis of variance and Fishers LSD (Snedecor, 1956). Contingency X^2 was used to statistically analyze the effect of surgery.

Results

Results of this experiment are summarized in Table 3. Four of 4 intact 70°F ewes in group 1, and 4 of 4 intact 90°F ewes in group 5 were pregnant after being exposed from the day of mating until slaughter of day 22. Five of 10 ovariectomized ewes in group 2 maintained at 70°F and 5 of 10 ovariectomized ewes in group 6 maintained at 90°F from the day of mating until slaughter on day 22 also possessed embryos; however, no heart beat could be observed in 2 of the embryos from ewes termed pregnant in the ovariectomized 90°F group.

None of 3 of the sham operated 70°F group and 1 of 3 of the sham operated 90°F group (groups 3 and 7 were pregnant at termination on day 22. Two of 4 ewes penned outside during the first run (July 31 through August 25, 1963) were pregnant at the termination day 22. Three of 4 ewes penned outside during the second run (Sept. 14 through Oct. 10, 1963) were pregnant at termination on day 22. Four of 4 ewes anesthesized with pentobarbital sodium but unopened (group 9) and maintained at 70°F from the day of mating until termination on day 22 were pregnant (Table 3).

Average embryo weights from the first run were 36.4, 57.9, 0 and 30.5 mg for groups 1, 2, 3, and 4, respectively. Average embryo weights from the second run were 53.5, 94.7, 34.2, and 49.8 mg, for groups 5, 6, 7, and 8, respectively (Table 3). The 94.7 mg average embryo weight of the ovariectomized 90°F group was significantly ($P < .05$) heavier than the other groups. The average embryo weight in the nembutal group was 40.8 mg. Average crown rump measurement of the embryos for groups 1 through 9 were 6.3, 7.3, 0, 6.3, 7.0, 8.1, 6.9, 7.1, and 6.3 mm, respectively. Group 6 was significantly ($P < .05$) larger than the other groups.

Average placental membrane weights were 164.0, 348.1, 0. and 140.0 mg for groups 1, 2, 3, and 4 (first run), respectively. Average placental membrane weights were 315.7, 503.0, 131.2, and 252.6 mg for groups 1, 2, 3 and 4 (second run), respectively. The ovariectomized 90°F group (group 6) was significantly ($P < .05$) heavier than all other groups. The average placental membrane weight of the ovariectomized 70°F group was significantly heavier than other groups except the intact 90°F group. The average placental membrane weights in group 9 was 414.5 mg. Eight of the 28 pregnant ewes were carrying twins.

Table 3. A summary of the effect of heat treatment on pregnancy in ovariectomized and intact ewes.

Group	No. ewes in group	No. ewes pg. at 22 days	No. ewes with 2 embryos	Avg. embryo wt. mg.	Avg. memb. wt. mg.	Avg. crown rump mm.
1. Intact 70°	4	4	1	39.7(3)**	164.0(4)	6.3(3)
5. Intact 90°	4	4	0	53.5(3)	315.7(4)	7.0(3)
2. OVX 70°	10	5	0	57.88(5)	348.1(5)	7.3(5)
6. OVX 90°	10	5*	2	94.65(4)	502.97(4)	8.1(4)
3. Sham 70°	3	0	0	-----	------	---
7. Sham 90°	3	1	1	34.2(1)	131.2(1)	6.9(1)
4. Outside 1st run	4	2	1	30.5(3)	140.0(3)	6.3(3)
8. Outside 2nd run	4	3	2	49.76(5)	252.6(5)	7.1(4)
9. Nembutal 70°	4	4	1	40.75(5)	414.5(5)	6.3(5)

*Heartbeat could not be detected at day 22 in embryos from 2 of 5 ewes.

**No. averaged. Data does not include dead embryos on those not intact.

Table 4. Chi square analysis of operative effect.

Group	No. ewes in group	No. preg. at 22 days
4. Sham 70°	3	0
8. Sham 90°	3	1
2. Ovx 70°	10	5
6. Ovx 90°	10	5*
9. Nembutal	4	4

*Heartbeat could not be detected at day 22 in embryos from 2 of 5 ewes.

Contingency X^2 significant $P < .05$ for above five groups (10.34).

Contingency X^2 not significant when nembutal group was excluded.

Discussion

Intact ewes exposed to an elevated ambient temperature from the day of mating until slaughter at day 22 maintained pregnancy in 4 of 4 cases. This was unexpected in view of results reported by Dutt et al. (1959), Dutt (1963) and Yeates (1953). Dutt (1963) observed 0% embryo survival when ewes were exposed to an environment of 90°F and 60% RH from the day of mating until day 20.

Dutt (1963) reported 40% of embryos survived when exposure was delayed until day 5. The difference in results reported by Dutt and others and results of this experiment are difficult to interpret, however, two possibilities must be considered in attempting to explain the discrepancy. One is the low relative humidity (35-45%) used in this experiment compared with 60% used by Dutt. The ewes used in this experiment were white face ewes purchased from the naturally warm climate of Texas. These multiparous ewes most likely had been previously culled to remove ewes which did not conceive after being bred during the hot months and thereby many of the heat susceptible individuals were removed.

Our original hypotheses in this experiment--does high temperature environment influence ovarian steroid production or utilization of these hormones--could not be tested because the intact heat treated group did not exhibit the expected detrimental effect of high environmental temperature. However, several noteworthy observations were made. Ten out of 20 ovariectomized ewes receiving 20 mg of progesterone and 4.0 ug estrone daily per 100 lbs. body weight maintained pregnancy. This is comparable to results reported by Foote et al. (1957) who used the same dosage but these workers ovariectomized 84 hours after breeding. Ovariectomy in this experiment was

performed approximately 36 hours earlier which is the reason for reducing the amount of estrogen and progesterone given the first two days after removal of the ovaries. It appears that since both groups of ovariectomized ewes maintained pregnancy in 50% of the cases, the 90°F environment was no more detrimental to these ewes than it was to the intact ewes.

In referring to the original hypothesis one might explain the similar rate of embryo survival in the 70°-ovariectomized group and the 90°F-ovariectomized group by merely stating that both groups received the same amount of estrogen and progesterone and the high temperature did not alter the effectiveness of the injected hormones. However, this is not a valid conclusion since there is no evidence in this experiment which suggest that the 90°F environment constituted a stressful condition.

A comparison of crown rump length and embryo weight indicated that embryos from ovariectomized-heat treated ewes were significantly ($P < .05$) larger and heavier than those in other groups. Placental membranes from ovariectomized heat treated ewes likewise were significantly ($P < .05$) heavier than in the other groups. Placental membranes from the intact 90°F group were significantly heavier ($P < .05$) than the intact 70°F group. Both embryo weights and crown rump measurements followed the same trend as placental weight and approached significance. These results tend to suggest that not only is the ovariectomy plus replacement therapy increasing embryonic development, but high temperature and ovariectomy may interact to increase embryonic development. Foote et al. (1957) also reported increased embryo weights in ovariectomized ewes given the same level of progesterone and estrogens as used in this experiment.

The cause of this increase in embryo weight may be related to an increased nutritional supply to the embryo possibly via greater development

of placenta or increased blood supply to the uterus (Kao et al., 1961). An equally plausible hypothesis is that hormone treatment and/or high temperature may have accelerated the process of implantation as Canivenc et al. (1953) found early implantation in lactating rats given progesterone locally in the uterine wall.

The embryo and placental membrane weights from ewes in the first phase of the experiment (groups 1-4) tended to be less than similar weights from ewes of the second phase of the experiment (Table 3). In addition, 3 of 4 of the ewes were pregnant in the outside environment during the latter phase compared with 2 of 4 in the first phase. One can conclude little on the basis of a single ewe; however, when the heavier embryo and placental weights observed in the latter phase of the experiment are considered, there is a suggestion of a seasonal effect.

The 16% embryonic survival in sham operated ewes compared with 50% of the ewes pregnant in the two ovariectomized groups suggest that cutting the mesovari or mechanical manipulation of the reproductive tract in the sham groups was more detrimental to embryo survival than complete ovariectomy plus steroid replacement (Table 4). Surgical trauma in both the sham and ovariectomized groups could have exerted a detrimental effect via some mechanism which resulted in a decreased rate of ovarian steroid synthesis and/or release in sham operated ewes. If this hypothesis were true the administration of progesterone and estrogen exogenously in the ovariectomized groups would compensate for the inadequacy caused by the operation itself and result in a more favorable rate of embryo survival in the later groups, when compared with the uninjected groups. Lamond (1963) also found laparotomy detrimental to embryo survival when performed early in gestation (day 4-5). Anesthetic alone did not appear to be involved in the low

embryo survival rates in the sham operated groups since 4 of 4 ewes maintained pregnancy when given only nembutal 48 hours after breeding.

EXPERIMENT 3

Methods and Materials

Two hundred forty immature female Sprague-Dawley rats were used to study the effect of two environmental temperatures (70° and 90°F) on the uterine weight response from two estrogens (estrone and estradiol-17 B). The 6 hour uterine weight assay of Astwood (1938) was employed. Rats were purchased from Hormone Assay Laboratory and arrived at 20 days of age. Rats were randomly assigned for assay either the following day or 2 days later depending on temperature. One 70° and 90°F replicate involving 120 rats was completed in two days. The order of temperature was then reversed to remove the effect of 1 day in age of rat and a second replicate of 120 rats was completed one week later.

Rats were placed 5 per cage and maintained in a 14 hour light regime at 75°F until the time of estrogen injection and then placed in a 6' x 6' x 6' walk-in environmental chamber (Tenney Engineering, Inc.) at either 70°F or 97°F $\pm$ 0.50. Relative humidity was maintained at 60% $\pm$ 6% at all times during test. Injections of either 0.0, 0.1, 0.2, 0.5, 1.0 or 2.0 ug estrone or 0.0, 0.01, 0.02, 0.05, 0.1 or 0.2 ug estradiol 17-B in 0.01 ml sesame oil were given subcutaneously via a microsyringe.

Six hours post injection rats were killed with chloroform and uteri excused, trimmed, blotted and weighed to the nearest 0.1 mg. The effect of dose and temperature was corrected for differences in body weight by analysis

of covariance (Snedecor, 1956). Differences in dose response were analyzed by stepwise regression (Efroymson, 1964).

Results

Mean corrected uterine weights of the 70°F environment were 28.1, 28.1, 36.1, 35.4, 36.2, and 36.2 mg for doses of 0.0, 0.01, 0.02, 0.05, 0.1 and 0.2 ug of estradiol 17-B, respectively (Table 5, column 1). Values at 97°F environment for the same doses of estradiol 17-B, respectively were 28.7, 26.5, 33.0, 37.8, 37.4, and 41.4 mg (Table 5, column 2).

The adjusted uterine weights at 70°F for the estrone groups were 26.8, 27.9, 32.7, 38.4, and 32.1 mg for doses of 0.0, 0.1, 0.2, 0.5, 1.0 and 2.0 ug, respectively (Table 5, column 3). The mean adjusted uterine weights of the estrone 97°F test were 26.0, 24.4, 26.2, 30.7, 33.3, and 36.2 mg for the respective estrone doses (Table 5, column 4). Differences in mean adjusted uterine weights of $\geq$ 5.2 mg are significantly different ($P < .05$). Only comparisons within a hormone are valid.

The average adjusted uterine weights (Table 6) were 29.09, 32.26, 33.92, and 33.04 mg for treatments estrone, 97°F, estrone, 70°F, estradiol, 97°F and estradiol, 70°F, respectively (LSD $\leq$ 2.1 mg). These values were arrived at by analysis of covariance as illustrated in Table 6. The general effect of estrone given to rats in 97°F environment was significantly less ($P < .05$) than the effect of estrone given to rats in the 70°F environment. There was no significant effect of temperature on the response of the estradiol.

The regression (b) of adjusted uterine weight on dose (1 ug of estrogen equal to 1 unit on X axis) for treatments estrone, 97°F, estrone, 70°F, estradiol, 97°F and estradiol, 70°F, were 5.729, 2.470, 51.956, and 23.933,

Table 5. The effect of high ambient temperature on estradiol 17-B or estrone induced increase in uterine weight using the 6 hour Astwood assay. Uterine weights represent the mean of 10 rats and are significantly (LSD P .05) different $\geq$ 5.2 mg. Only comparisons within a hormone are valid.

Estradiol 17-B			Estrone		
Dose (ug)	70°F Uterine wt.(mg)	97°F Uterine wt.(mg)	Dose (ug)	70°F Uterine wt.(mg)	97°F Uterine wt.(mg)
0.00	28.1	28.7	0.0	26.8	26.0
0.01	28.1	26.5	0.1	27.9	24.4
0.02	36.1	33.0	0.2	32.7	26.2
0.05	35.4	37.8	0.5	35.7	30.7
0.10	36.2	37.4	1.0	38.4	33.3
0.20	36.2	41.4	2.0	32.1	36.2

Table 6. Analysis of the general effect of estrone or estradiol 17-B on the weight of the uterus of the immature rat. Uterine weights were adjusted for differences in body weight of the test animal by analysis of covariance.

Treatment		$\bar{X}$	$\bar{Y}$	$\bar{X}-\bar{\bar{X}}$	$b(\bar{X}-\bar{\bar{X}})$	$\bar{Y}$ adj
Estrone	97°F	47.883	29.842	1.321	.752	29.090*
Estrone	70°F	45.433	31.612	-1.129	-.643	32.255
Estradiol	97°F	47.433	34.413	.871	.496	33.917
Estradiol	70°F	45.500	32.700	-1.063	-.605	33.035

$\bar{X}$ = group mean body weight (gm)

$\bar{Y}$ = group mean uterine weight (mg)

$\bar{X}-\bar{\bar{X}}$ = group mean body weight - overall mean (46.563 gm)

$b = \frac{xy}{x^2}$ = .5695 common regression. See Table 7, b.

$\bar{Y}$ adj = $\bar{Y}$ - b ($\bar{X}-\bar{\bar{X}}$) = mean uterine weight after adjusted for differences in body weight.

*Significantly different ($P < .05$) from general effect of estrone given to rats in 70° environment. (LSD $\geq$ 2.1 mg)

Table 7. Summary of the effect of temperature (70° and 97°F), hormone (estrone and estradiol-17 B) and the interaction of temperature and hormone on adjusted uterine weight. Data analyzed by covariance.

Source	df	x^2	xy	y^2	df	$d^2y{\cdot}x$ +	M_s	z	b
Temperature (T)	1	288.20	-3.72	.04					
Hormone (H)	1	2.20	-32.54	480.53					
T x H	1	4.01	-27.00	182.01					
Error	236	4240.65	2415.24	9829.04	235	8453.45	35.97		.5695
Total	239	4535.06	2351.98	10491.62					
T + Error		4528.85	2411.52	9829.08	236	8544.99		2.54	
Test T					1	91.54	91.54		
H + Error		4242.85	2382.70	10309.57	236	8971.49			
Test H					1	518.04	518.04	14.40**	
T x H + Error		4244.66	2388.24	10011.05	236	8667.31			
Test T x H					1	213.86	213.86	5.95*	

*P < .05

**P < .01

+ $d^2y{\cdot}x = y^2 - (xy)^2/x^2$

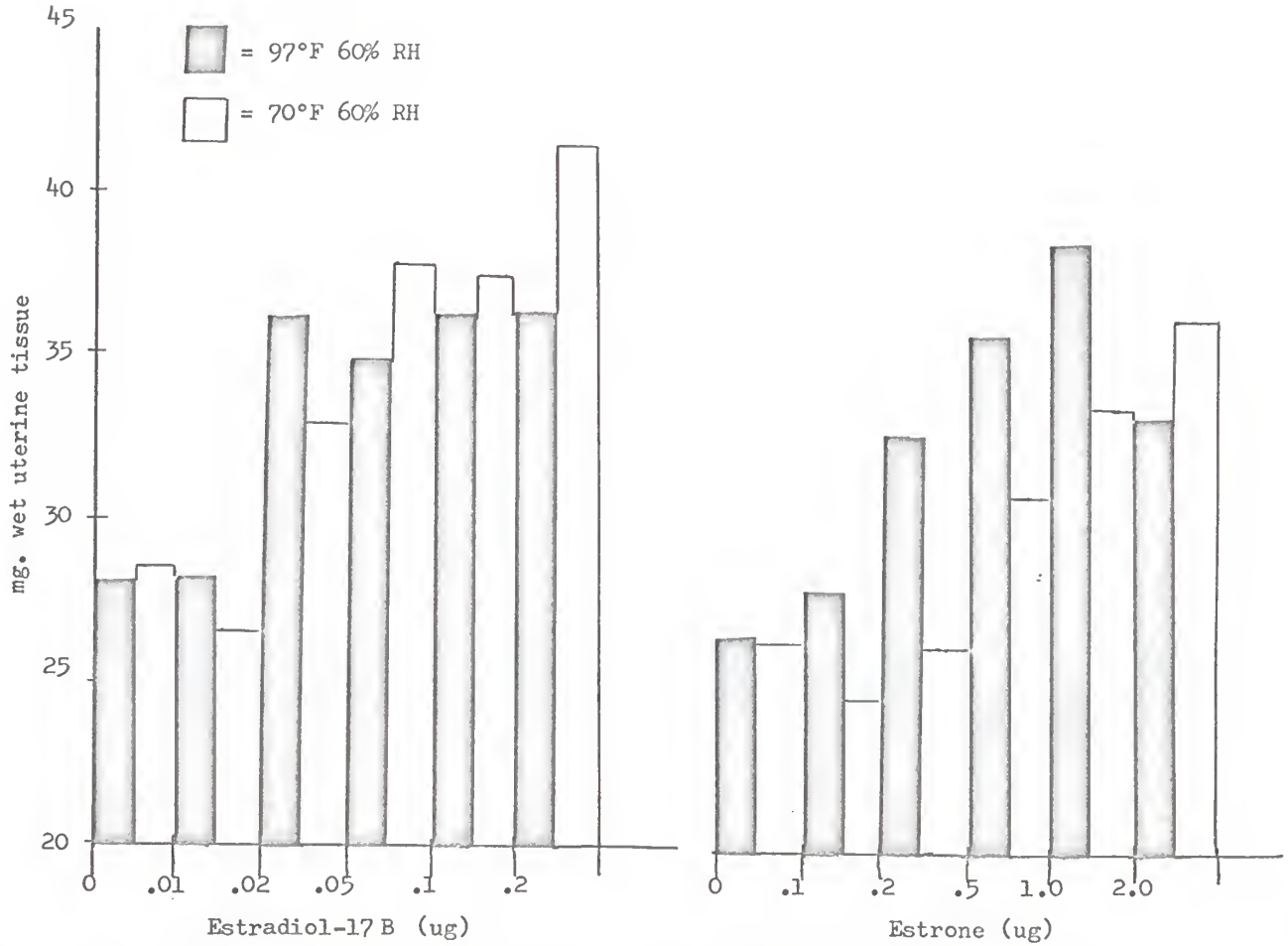
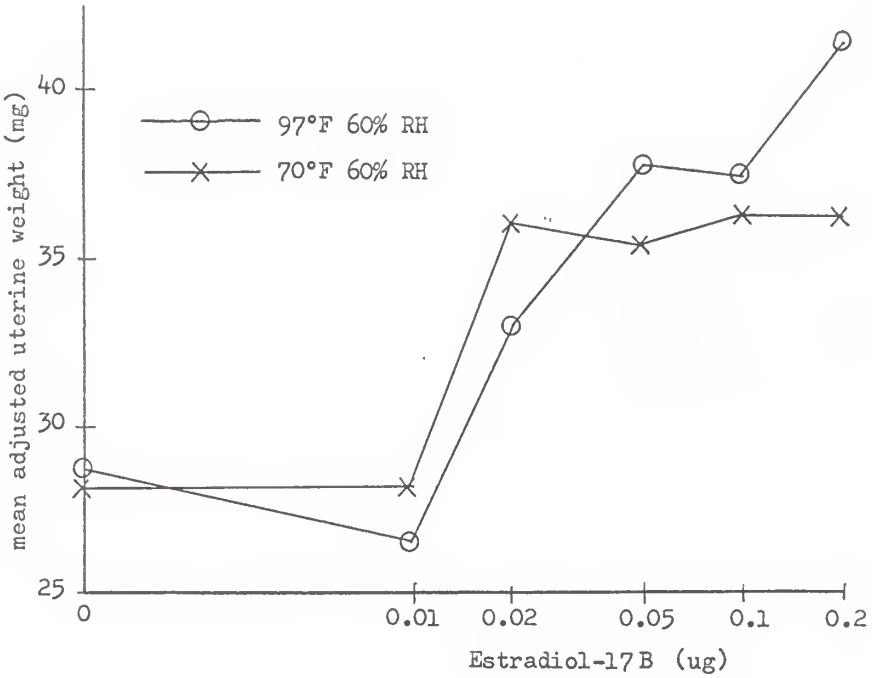


Fig. 1. Uterine wt. response to estrone and estradiol at two temperatures. (Uterine weights were adjusted for differences in body weight by analysis of covariance.)



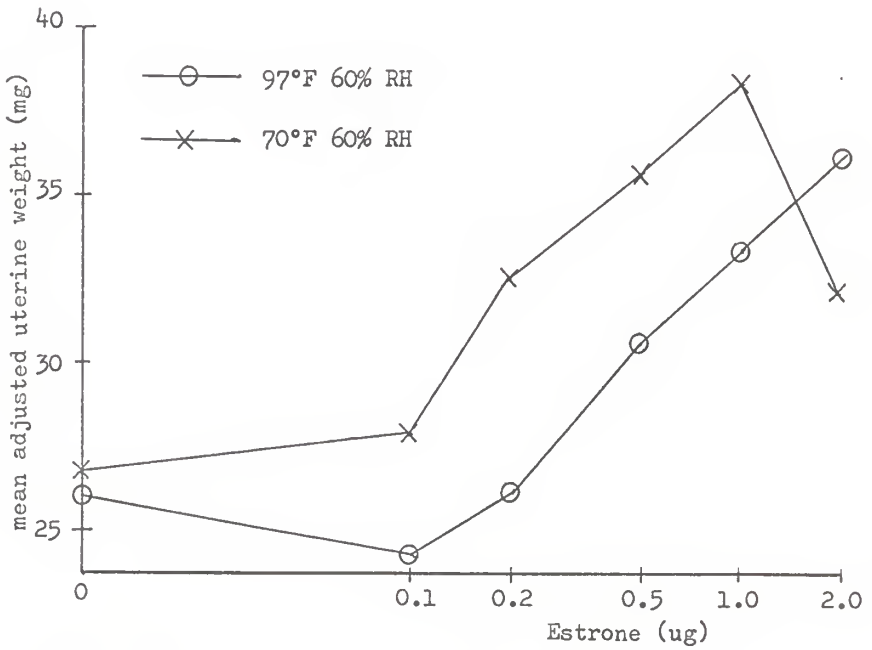
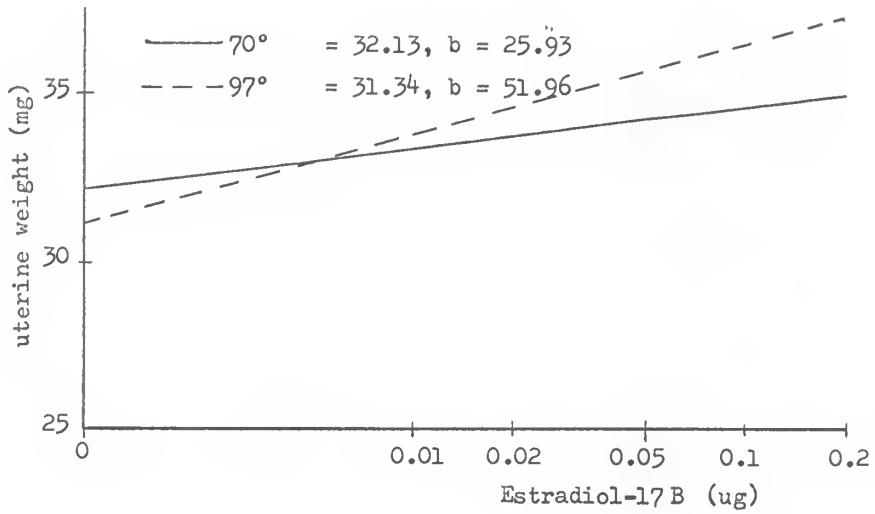


Fig. 2 and 3. Dose response curves of estrone and estradiol-17 B. Uterine weight is plotted vs. log of the estrogen dose at 70° and 97°F.



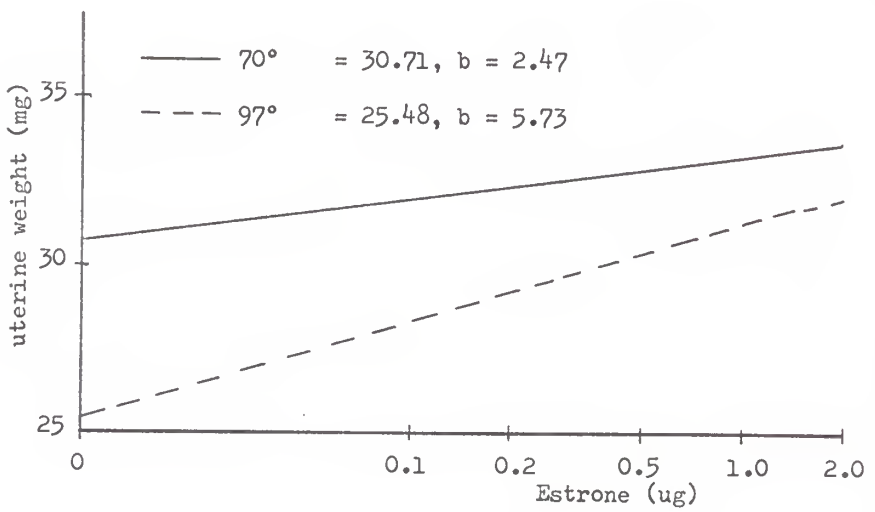


Fig. 4 and 5. Shows the calculated regression of adjusted uterine weight on log of estrogen dose. The origin ( ) and slope (b) is given for each treatment in the respective graph.

respectively. These regression coefficients (b's) increase which indicates ($P < .05$) that all four treatments significantly increased the weight of the uterus of the test animals.

The calculated origin (α) in the estrone tests was 30.71, 25.48 for the 70°F and 97°F run, respectively. The actual origins were 26.8 and 26.0 mg respectively. The calculated origin for estradiol (α) was 32.13 and 31.34 for temperatures run 70° and 97°F, respectively. The actual origins were 28.1 and 28.7 mg respectively. This discrepancy indicates that the response was not linear.

According to the comparison of regression coefficients between estrone and estradiol at the same temperature, the ratio of effectiveness of estrone: estradiol was 1:9.68 at 70° and 1:9.07 at 97°, however, not all doses of the two hormones appeared to fall within the range of linear response (see Fig. 2 and 3). The difference between calculated origins (Fig. 4 and 5) and the actual origins also illustrate this point. Consequently, a comparison of effectiveness of the two hormones is not entirely valid.

Discussion

Astwood (1938) demonstrated that the uterus of the immature rat rapidly increases in weight following the injection of estrogen. This phenomenon was used in this experiment not because it represents the most accurate or sensitive method to assay estrogenic activity, but because a short term procedure was needed whereby the effect of high environmental temperatures on the potency of known estrogen doses could be compared on a biological basis.

The purpose of this experiment was two-fold. One part was to confirm preliminary experiments which indicated that the increase in wet weight of

the uterus of immature rats given estrone was greatly curtailed by concomitant high temperature stress. The second objective was to determine if high ambient temperature might alter the metabolic conversion of estrone to estradiol by the test animal. This second objective was based on evidence of Jensen and Jacobson (1962) that indicates estrone must be metabolically converted to estradiol before the estrogenic response in the uterus can be produced.

It can be seen in Fig. 1 and Fig. 3 that the response of the uterus of rats given estrone at the 97°F environment was less than that observed in rats maintained at the 70°F environment. This difference was significant ($P < .05$) as seen in Table 6. In contrast there appeared to be little effect of high temperature on the increase in uterine weight when estradiol-17 B was given. This can be seen in Fig. 1 and 2. In fact there was a tendency toward heavier uteri at the 97° environment with estradiol (line 3 and 4, Table 6). It would appear that high environmental temperatures had no detrimental effect on the mechanism responsible for the increase in wet weight of the immature rat uterus when estradiol was injected. However, when estrone was administered to rats subjected to high environmental temperature, the potency of this estrogen was significantly reduced as compared with the control temperature groups (Fig. 1 and line 1 and 2 of Table 6). These results suggest that estrone and estradiol do not act in an identical manner on the uterus of the immature rat. This conclusion is supported by results of Jensen and Jacobson (1962) who found that following injection of radioactive estrone only labeled estradiol could be recovered from the rat uterus. From their data they concluded that estradiol was the form of estrogen active in eliciting uterine growth and that estrone could produce the estrogenic affect only after being converted to estradiol.

Stone (1963) and Stone et al. (1963) also have shown differences in the mechanism of estrone and estradiol induced growth in the uterus of the mouse.

It is not likely that the decreased response noted in the estrone 97°F groups compared with estrone 70°F groups was due to a decreased availability of body water at the high temperature. If this were the only reason for the difference, the estradiol trial would exhibit the same trend as the estrone trial. The uterine weights of the two groups receiving only sesame oil were also similar in both the 70°F and 97°F trial. It is not known why the weights of the uteri in the low doses of both the estrone and estradiol in the high temperature trials tended to be lower than that observed in the 70°F trial. However, this fact and the apparent refractiveness of the highest dose of estrone of 70°F (Fig. 2) contributed to the significantly different ($P < .05$) calculated point of origins (α) while the actual values for sesame oil controls at the two temperatures were in fact very similar in both the estrone and estradiol trial. This difference makes a test of parallel response meaningless and also makes a comparison of the regressions of dose of estrogen on adjusted uterine weight invalid. It was hoped that the ratio of effectiveness of estrone and estradiol at the two temperatures could be compared, but for the same reasons as mentioned above, this test is not entirely valid.

Since high temperature did have a significant effect on the effectiveness of estrone but had no apparent effect on estradiol, one might speculate that the reason for this difference is that high temperature in some manner affects the conversion of estrone to estradiol. Since estradiol and not estrone is thought to be the form of estrogen active in producing the increase in uterine weight (Jensen and Jacobson, 1962), no reduction in potency of estradiol given at the 97°F environment would be expected even if

the mechanism which converts estrone to estradiol was affected by high temperature stress.

If high temperature stress affects the mechanism which converts estrone to estradiol, its route of action could be direct, by an elevated body temperature which could interfere with the conversion; or indirect, because of an elevated production of adrenal steroids or some other inhibitory process.

Several experiments have been conducted which indicate that adrenal corticoids can prevent estrogenic responses when the estrogen and the corticoid are given concomitantly. Szego (1948), Spaziani et al. (1948, 1959a, 1959b), Edgren et al. (1961) and Spaziani (1963) have reported that cortisol inhibits the increase in weight of the rat uterus following estrogen administration. Also high doses of corticoids prevent the response to estradiol treatment (Spaziani et al., 1958, and Spaziani 1963).

A more valid test of the role of the adrenal in altering the conversion of estrone to estradiol would be found with adrenalectomized test animals given estrone and subjected to thermal stress.

SUMMARY

Embryo survival was not significantly affected by exposing rats to 97°F environment from the day following mating until day 8. The number of corpora lutea counted at day 18 was significantly increased by the heat treatment and ovariectomy 60 to 68 hours after mating plus daily replacement therapy of 4 mg progesterone and 1 ug estrone, successfully maintained pregnancy at a rate not significantly different from intact rats. Embryo survival was not significantly different between intact, 70°F and ovariectomized, 75°F females or intact, 97°F and ovariectomized, 97°F females. The 18 day embryo and placental weight was increased by ovariectomy.

No difference in the rate of embryo survival was noted between intact ewes housed at 70°F or 90°F either. The same was true for ovariectomized ewes receiving estrogen and progesterone. Sham operation appeared to be more detrimental to embryo survival than ovariectomy plus replacement therapy. Larger embryos and heavier placenta were found in the pregnant ovariectomized ewes.

Immature female rats given estrone and exposed to 97°F environment during a 6 hour biological estrogen assay exhibited a significantly reduced uterine response when compared with the uterine response of rats given estrone at 70°F. There was no significant difference in uterine responses at the two temperatures when estradiol-17 B was given. Estrone at 70°F produced a similar uterine response as estradiol (70° or 90°F) when given in a ratio of 10 to 1.

These three experiments were all designed to study the mechanism by which high temperature stress affects reproductive performance in the ewe and rat. The first two experiments primarily investigated the possibility that high temperature stress affects production of ovarian steroids, which in turn affects embryo survival. No positive evidence for this possibility was obtained. The third experiment yielded information which suggested that high temperatures may decrease the effectiveness of estrone in heat stressed rats. Since estrogen is necessary for several of the reproductive processes susceptible to high temperature stress, it is likely that a decreased utilization of estrogen may be responsible for a part of the low embryo survival noted in heat stressed animals.

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ENDOCRINE-CLIMATIC RELATIONSHIPS THAT INFLUENCE REPRODUCTIVE TRAITS IN EWES AND RATS

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Rats and ewes were used in an attempt to study the mechanism responsible for early embryonic mortality noted in farm animals subjected to high environmental temperatures. The hypothesis under test was that thermal stress results in either altered production or utilization of ovarian steroids. Both rats and ewes were subjected to 97°F and 90°F, respectively, soon after mating. The female rats and ewes were ovariectomized 3 and 2 days after mating, respectively, and pregnancy was maintained with 4 mg progesterone and 1 ug estrone per rat, or per 25 lb. body weight of the ewe. In the absence of endogenous ovarian steroids all females, irrespective of environment, received the same quantity of hormones and any effect of temperature would therefore be due to something other than variations in production of ovarian steroids. In both experiments temperature failed to significantly reduce embryo survival in either intact or ovariectomized groups. It was concluded that temperature was no more detrimental to ovariectomized females than intact animals, however, the effect of temperature on ovarian steroidogenesis is still not clear. It was noted in both experiments that the embryo and placental weights from ovariectomized dams tended to be heavier than from intact females.

The effect of high temperature on utilization of estrone or estradiol-17B was also studied by use of the Astwood 6 hour uterine weight response assay. The effectiveness of six levels of both hormones was tested at 70° and 97°F. High temperature had no effect on the uterine response to estradiol but significantly reduced the response to estrone. It was concluded that high temperature hindered the conversion of estrone to estradiol which is thought to be a prerequisite for estrogenic activity of estrone in the uterus of the rat.