THE INFLUENCE OF HIGH AMBIENT TEMPERATURE PRIOR TO AND DURING ESTRUS UPON OVULATION RATE AND EMBRYO SURVIVAL IN GILTS

By

STEPHEN R. PAYER

Bachelor of Science

California State Polytechnic College

Pomona, California

1970

Submitted to the Faculty of the Graduate College
of the Oklahoma State University
in partial fulfillment of the requirements
for the Degree of
MASTER OF SCIENCE
May, 1971

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Thesis Approved:

E. J. Turman
Thesis Adviser

Milton Wells

Irvin T. Omtvedt

D. D. Durham
Dean of the Graduate College

ACKNOWLEDGMENTS

The author expresses his sincere appreciation to Dr. E. J. Turman, Professor of Animal Science, and Dr. I. T. Omtvedt, Professor of Animal Science, for their counsel and guidance during the course of this study and in the preparation of this thesis.

Further appreciation is extended to Dr. Robert Morrison, Professor of Mathematics and Statistics, and Mr. Don Holbert, Graduate Assistant, Department of Mathematics and Statistics, for their assistance in the analyses of the data.

Special recognition is extended to John Magee who transported and cared for the experimental animals and assisted with collection of the data.

The author is grateful to Ralph's Packing Company, Perkins, for making the reproductive tracts available.

Additional thanks are extended to fellow graduate students in the Department of Animal Sciences and Industry for their assistance during the course of this study.

A special thanks goes to the author's wife, d'Aulan for her patience and understanding during the course of this study.

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CHAPTER I

INTRODUCTION

Economic conditions within the swine industry have resulted in the development of large-scale confinement operations. To make these facilities economically feasible, operators are seeking to maximize their return on their investment by farrowing during all months of the year.

However, swine producers and research scientists have reported that sows bred during the hot summer months farrow smaller litters than those bred during the cooler months. This decrease in litter size appears to be the result of high ambient temperature stress exerting its effect during some particular phase of the reproductive cycle.

The detrimental effect of high temperatures on swine reproductive performance may be directly upon one or more reproductive mechanisms. Ova formation, ovulation, fertilization and subsequent cleavage, implantation or the oviducal and uterine environment might possibly be affected. Indirectly, the heat stress syndrome could be mediated via lowered feed consumption or a general endocrine imbalance within the animal. However, since general body metabolism is so intimately related with the reproductive processes it becomes difficult to implicate one particular cause without considering many others.

This study was initiated on June 15, 1970, to investigate the effects of high ambient temperature upon gilts for the period of the cycle five days prior to breeding through two days after breeding, using ovu-

lation rate, fertilization rate, and embryo survival as criteria. This investigation was completed November 16, 1970, and the results are presented herein.

CHAPTER II

LITERATURE REVIEW

Throughout the literature, there is much variation among reports of the effects of high temperature on reproductive performance in general and on embryonic survival in particular. This variation can be attributed to the inherent differential response between species, the stage of the estrual cycle or pregnancy during which the animal is exposed to heat stress, the length of the exposure period, and the degree of stress applied to the animal.

Laboratory Animals

Macfarlane et al. (1957) reported fetal loss and reabsorption of up to 58% among pregnant rats exposed to 35°C. during pregnancy. Laparotomies were carried out on groups of rats on the sixteenth day of gestation. Acclimation to 35°C. for 2 to 10 weeks prior to mating significantly reduced fetal loss from a mean of 31% for the unacclimated rats to a mean of 10% for the acclimated. The latter figure is comparable to the loss (7%) in controls held at 22°C.

Aldred et al. (1961) subjected virgin female mice to 104°F. for 1, 3, or 5 hours on the day following mating or for 5 hours on day 1 and 2 following mating. At autopsy on day 17 of pregnancy it was observed that as duration of exposure to heat stress was increased, embryo loss also increased (control - 14%; 1 hour - 14.5%; 3 hours - 16.8%; 5 hours -

19.3%; 5 hours day 1 and 2 - 32.5%). Embryo loss following exposure only on day 2 was 9.8%, less than that observed for controls (14%).

Fernandez-Cano (1958a) reported that an increase of 5^{o}F. in body temperature of female rats as a result of exposure to 103^{o}F. for 5 hours on 2 consecutive days during early pregnancy (day 1 and 2, day 3 and 4, day 6 and 7, or day 10 and 11) increased embryonic degeneration mainly prior to implantation.

In a study similar to the previous, Fernandez-Cano (1958b) utilized bred female rats adrenalectomized prior to exposure to 103^{o}F. on the third and fourth day after successful mating. He reported no significant differences in embryonic degeneration between controls and heat-stressed females at 15 days of gestation (6.2% vs. 6.2%, respectively). The author suggested that an adrenal-pituitary reaction resulting in increased cortical hormones may be responsible for the embryonic degeneration observed in this experiment.

Howarth (1969), in a study of heat-stressed adrenalectomized does, observed that removal of the adrenal glands did not improve fertility. It was observed to depress fertilization rate and increase the number of morphologically abnormal ova.

Elliot et al. (1968) presented a report on the development of ova, in vitro, following recovery from heat-stressed female mice. On the day a postcopulatory plug was observed following mating, females were subjected to a 34^{o}C. environment for 24 hours and then returned to a 21^{o}C. chamber. Ova recovered from heat-stressed females 48 to 54 hours after mating were incubated in vitro. Significantly fewer ($P < .01$) 8-cell ova were recovered from stressed females (33.6%) than from controls (51.1%). Significantly fewer ova from heat-stressed females con-

tinued to develop after 3 days incubation as compared to controls (25.4% vs. 41.4% respectively, $P < .01$).

At autopsy on day 10 of gestation, it was observed that pre- and post-implantation loss of embryos significantly reduced overall embryo survival in heat-stressed females as compared to controls (41.1% vs. 71.8%, respectively, $P < .01$).

Shah (1956) utilized a reciprocal ova transfer technique to demonstrate the effects of heat stress upon embryo mortality in the rabbit. Six-day old blastulae were transferred from does maintained at 96°F. for 6 days following coitus to recipient does maintained at normal room temperatures. Only 15 (35.7%) of these embryos developed into normal young. Similar results (37.5%) were obtained when donor and recipient were held at the same temperature (70°F.). However, when 66 embryos were transferred from low temperature (70°F.) does to 9 high temperature (96°F.) recipients, entire litters were resorbed in 8 of the recipient does. The author concluded that the heat stress affected the embryo indirectly via some alteration in the physiology of the maternal tissue, rather than directly upon the embryo itself.

To study the effects of <u>in vitro</u> culture temperatures upon viability of rabbit ova, Alliston and Ulberg (1963) transferred 4- and 8-cell fertilized ova to synchronous pseudo-pregnant females. For a control, ova were cultured for 6 hours at 38°C. which was normal rectal temperature. Heat-stressed ova also were cultured for 6 hours, but at 40°C. which corresponded with the rectal temperature of rabbits maintained at an ambient temperature of 32°C. Autopsy at 12 days post-coitum revealed no significant differences among rates of embryo survival resulting from implantation of stressed and control cultured ova. The authors concluded

that adverse effects of increased maternal temperature directly upon ova (and therefore embryo survival) may be limited to the period prior to completion of the first cleavage following fertilization.

Further work by Alliston et al. (1965) was conducted in a similar experiment except one- and two-cell fertilized ova were transferred. Significant ($P < .01$) differences were demonstrated in embryo survival 12 days post-coitum resulting from control-cultured one-cell ova (95.5%) as compared to stress-cultured one-cell ova (74.3%). This difference was not demonstrated using two-cell ova cultured after completion of the first cleavage. The implantation rates of 1- and 2-cell stressed and control ova were comparable. Therefore, the authors concluded that effects upon the early rabbit embryo (fertilized one-cell) by increased maternal body temperature may not become apparent until later stages of embryonic development, subsequent to implantation.

Howarth et al. (1965) studied the effects of elevated ambient temperature ($32^{\circ}C.$) upon rabbit sperm capacitated for 6 or 8 hours in the uteri of stressed females. The sperm were then transferred to females maintained at $21^{\circ}C.$ which had been mated to vasectomized males 10 hours prior to sperm deposition. Sperm fertilizing capacity was not significantly altered as a result of capacitation at increased temperature. There was a significant ($P < .01$) decrease in pre-implantation embryo survival indicative of an effect of elevated ambient temperature, mediated via the uterine environment, on sperm.

In a discussion of the literature reviewing embryonic death caused by an adverse environment on spermatozoa or ova, Ulberg and Burfening (1967) stated that the stress of increased temperature, whether on the sperm prior to fertilization or on the ovum immediately after fertiliza-

tion, causes the resulting embryo to die at some later point in its development. Thus, the damage to the embryo is caused in early development but becomes apparent only at some later stage.

Rich and Alliston (1970) observed the effects of temperature changes on rabbits acclimated to 21.1°C. or 32°C. for 18 days prior to artificial insemination. They reported no significant difference in embryo survival rates between acclimated and non-acclimated females following slaughter 12 days after insemination.

Cattle

Stott and Williams (1962) studied the effects of high seasonal temperatures upon breeding efficiency in Holstein heifers during the summer months in Arizona. Conception rates declined from 61.5% to 31.0% as daily temperatures increased 15°F. Of 111 heifers bred during August only 19 (17.1%) were pregnant at 35 to 41 days. They concluded that the critical period for embryonic loss as a result of high ambient temperature is during the first 35 days of pregnancy. However, a low rate of fertilization due to elevated temperatures at or near breeding was also associated with the low seasonal breeding efficiency observed in this study.

Ragsdale *et al.* (1948) reported that two Holstein cows simultaneously aborted 4½ and 6 month old fetuses 2 days after exposure to 100°F. for 27 hours.

Hillin and Rupel (1960) reported definite seasonal differences in the number of services required per conception in a study comparing Holstein and Jersey cattle. Holstein cattle appeared more severely affected by increased summer temperatures. Services required for concep-

tion for heifers first bred during June through October compared to those bred first during the remainder of the year, were 3.12 and 2.11 for Holsteins and 2.13 and 2.09 for Jerseys, respectively.

Further work studying seasonal reproductive performance of dairy cattle was reported by Poston et al. (1960). From the 10-year breeding records of six herds of dairy cattle located in North Carolina they reported an increase in the percentage of returns to service from a minimum of 38% in January to a maximum of 56% in August. From an examination of 2,541 individual records they reported a maximum calving interval of 422 days for cows calving in May, after which there was a monthly decline to a minimum interval of 397 days for cows freshening in October.

Sheep

Dutt et al. (1959) reported that fertilization rate and embryo survival are adversely affected by high ambient temperature. One hundred twenty Northwestern ewes were utilized in the study involving exposure to 90°F. for heat-stressed ewes from 12 days after the onset of the previous estrus to 3 days post-breeding at the following estrus. Of ova recovered from control ewes held outside and stressed ewes, 92.6% and 51.9% were cleaved, respectively. They noted fertilization rates of 64% for shorn ewes and 40.7% for unshorn ewes maintained at 90°F. Control ewes ovulated 3.7% abnormal ova compared to 32% (shorn) and 55.6% (unshorn) for heat stressed ewes. Embryo loss was determined by laparotomy 32 to 35 days after breeding. Loss for controls totaled 4% compared to 91.7% for ewes exposed to elevated temperatures prior to breeding. This loss was not as dramatic (15.4%) when ewes were first exposed to the heat stress 8 days after breeding.

To more specifically determine a critical period for embryo survival Dutt (1963) studied the effects of heat stress at time of breeding or shortly thereafter. Estrus ewes were exposed to 90°F. immediately after breeding (0-day group) or at intervals of 1, 3, and 5 days post-breeding. Fertilization rate was 69.2% for 0-day ewes, compared to 96% for controls (no significant difference). Control ewes had 3.7% abnormal ova compared to 46.2% and 30.8% for 0-day and 1-day groups, respectively, a significant difference ($P < .01$). Embryo loss was greater in the 0-day and 1-day ewes (100% and 77.8%, respectively) as compared to that loss observed in the 3- and 5-day groups (61.5% and 65.4%, respectively). All were significantly greater than controls, (19.2%, $P < .01$). Combined mortality in 0- and 1-day ewes was greater ($P < .05$) than that of 3- and 5-day ewes. The author concluded the critical period for survival of the sheep zygote is during the initial stages of cleavage while in the oviduct.

Alliston and Ulberg (1961) reported that damage to the embryo due to high ambient temperature (90°F.) occurred prior to 3 days after mating. Utilizing an embryo transfer technique, they observed that when both donor and recipient ewes were maintained at 70°F., 56.5% of the embryo transfers were successful. However, when donor and recipient were held at 70°F. and 90°F. respectively, less than 10% of the embryo transfers were successful.

Woody and Ulberg (1964) exposed ewes to 90°F. from 12 or 14 days after their last estrus until pregnancy-verifying laparotomy at 25 to 30 days post-estrus or until they returned to estrus. More control ewes were pregnant at 25 to 30 days following mating than heat-stressed ewes (71.4% vs. 35.0%, respectively, $P < .025$). When single unfertilized ova

were transferred from stressed donors (90°F.) to control recipients (70°F.) mated prior to transfer, 46.7% of the ova transferred resulted in pregnancies, compared to 35.3% for control donor and control recipient transfers. However, when fertilized ova were transferred from 70°F. donors to 90°F. recipients, 21.4% of the transferred ova resulted in pregnancy, compared to 58.8% for control to control transfers. Data from ewes shifted from one temperature to the other at the end of estrus indicated that most of the decrease in fertility attributable to high ambient temperature occurs by the end of estrus.

Thwaites (1969) reported that continual exposure to heat stress resulted in an embryo loss much greater than that observed from ewes in a diurnally variable environment. In one experiment, bred Merino ewes were exposed daily for 15 days to an 8-hour period of 106°F. followed by a 16-hour period of 95°F. Control ewes were maintained outside (46°F. to 63°F.) and experienced an embryo loss of 22.2% compared to 35.3% for the diurnally stressed ewes. In a second experiment shorn and unshorn ewes held at constant 96.5°F. lost 66.6% and 100% of their embryos, respectively, while variably stressed ewes lost none and 33% respectively. It is apparent then, that a diurnally flucuating environment affects the ewe differently than continuous exposure to heat stress.

Work supporting that of Thwaites (1969) was reported by Rich and Alliston (1970). Nonsignificant differences in percent of abnormal ova, fertilization rate, and embryo survival were observed between control ewes (constant 21.1°C.) and ewes subjected to variable heat stress (maximum 32.2°C., minimum 21.1°C.).

Further work by Thwaites (1970) involved the use of progesterone, thyroxine, or cortisol therapy in an attempt to study the effect on

embryo mortality. Heat stress did not significantly affect histology, size, or weight of corpora lutea. Progesterone therapy did not enhance embryo survival in stressed ewes. Thyroid epithelial cell height was significantly reduced by heat stress, presumably being evidence of decreasing thyroid secretion. However, thyroxine therapy did not affect survival of embryos in control or stressed ewes. Cortisol secretion rate was not increased by heat stress and cortisol therapy did not increase embryo loss. It was suggested by the author that changes in the luminal fluids of the oviducts and uteri of heat-stressed ewes are implicated in early embryonic mortality, even though no heat-induced changes in the uterine endometrium were noted in this study.

Heat stress may manifest its effects in other ways. Yeates (1958) studied the effects of high ambient temperatures during pregnancy on fetal dwarfism in sheep. Seven Merino ewes were placed in a hot chamber following service and maintained there until just prior to lambing. Ewes were exposed to temperatures of 112°F. for 7 hours and 90°F. for the remainder of the day. Four of the seven ewes lambed, producing lambs weighing an average of 2 pounds 13 ounces less than lambs born to unheated ewes. The authors suggested either a placental effect, reduced uterine blood supply, pituitary insufficiency or adrenal steroid stress as possible causes of dwarfing in sheep.

Swine

Heitman and Hughes (1949) reported that when hogs weighing more than 200 pounds were exposed to a temperature of 96°F. and 30% humidity, body temperature increased 2.5°F. and respiration rate doubled.

Further work by Heitman et al. (1951) demonstrated that the pregnant

sow was more susceptible to high temperatures than the non-pregnant female. When exposed to 98°F., an open sow showed a respiration rate of 64 per minute, while a pregnant sow of similar age and weight, showed a respiration rate as high as 186 per minute. They concluded that extremely high temperatures (rectal temperature 113°F.) will kill the sow prior to causing death of the litter and subsequent abortion. Daily feed consumption for heat-stressed sows was reported as 50% of normal.

Whatley et al. (1957) studied the value of cooling pregnant females with water sprinklers during June, July and August. Maximum air temperatures exceeded 90°F. during 77 days of the trial. Sprinkling pregnant sows resulted in 2.35 more pigs farrowed per litter compared to non-sprinkled females. Rectal temperatures were significantly higher ($P < .01$) for the unsprinkled sows (103.8°F.) than for sprinkled sows (101°F.). Sows not given the benefit of sprinkling did not readily consume their morning and afternoon feed, as did the sprinkled females.

Warnick et al. (1965) reported that 28 gilts exposed to 60°F. from the tenth day following first estrus to 25 post-breeding at second estrus had 1.9 more embryos at slaughter (25 days following breeding) and 1.1 more corpora lutea than 29 gilts maintained at 90°F. for the same period. Average crown rump lengths of embryos were 17.69 mm and 17.20 mm for 60° gilts and 90° gilts, respectively. It was emphasized that although cooling gilts soon after breeding may increase embryonic survival, the gilt otherwise tolerates a temperature of 90°F. with no grossly adverse side effects.

Tompkins et al. (1967) presented data indicating that elevated temperature detrimentally affects embryonic survival, if the sow is exposed to heat stress during the first 5 days of gestation.

Following a 3-year study involving 240 sexually mature Duroc gilts, Teague et al. (1968) concluded that ovulation rate, as measured by number of corpora lutea 25 days post-breeding, was significantly decreased ($P < .05$) as dry-bulb temperatures increased to 33.3°C. However, differences in number of live embryos were not significantly associated with increasing temperature. Gilts were exposed to heat stress for one estrus cycle prior to breeding and for the first 25 days of pregnancy.

Edwards et al. (1968) studied the effects of high ambient temperature (38.9°C. for 17 hours daily - 32.2°C. for remaining 7 hours) prior to breeding and during early gestation. When gilts were exposed for one cycle prior to breeding, they apparently adapted to increased temperature as ovulation rates and embryonic survival were comparable to that of controls. Gilts stressed 1 to 15 days post-breeding had fewer ($P < .01$) viable embryos than controls. Exposure to heat stress 15 to 30 days post-breeding did not affect embryo survival as compared to controls, however, the embryos from stressed gilts tended to be smaller.

Omtvedt et al. (1971) reported that pregnant gilts tend to be most resistant to high ambient temperatures during mid-pregnancy (53-61 days post-breeding). The most critical periods for embryo survival in this study were early (0 to 16 days) and late (102 to 110 days) pregnancy. Gilts stressed during early pregnancy had fewer ($P < .01$) and smaller viable embryos at slaughter (30 days post-breeding) than did controls. Gilts maintained in the hot chamber from 8-16 days following breeding had fewer ($P < .01$) live embryos than 0 to 8 day gilts (6.9 vs. 11.4 respectively). It has been reported by Nalbandov (1964) that blastocysts float free in the uterus until between days 11 and 20 of pregnancy, during which time implantation occurs. It would seem then, that the im-

plantation period is the most critical for embryo survival in early pregnancy. Analysis of individual rectal temperature data in this study was made to determine if reproductive performance was associated with degree of rectal temperature elevation during heat stress or degree of acclimation while in the chamber. It was concluded that rectal temperature data is not an adequate predictor of the effect of heat stress on reproductive performance.

In a study with 80 cycling gilts, d'Arce et al. (1970) reported that 8 gilts exposed to 28.9°C. from day 16 of the estrus cycle to 2 days subsequent to the following estrus, (at which time they were slaughtered), had an average of 11.9 ± .08 corpora lutea. However, ovulation rates compared across different periods of exposure to stress were comparable. Estrous cycle length was not increased significantly due to exposure to heat stress. Gilts exposed to heat stress during the preovulatory phase exhibited an individual variation in luteal development that may be indicative of prolonged or delayed ovulation.

Ova Recovery

Perry and Rowlands (1962) reported that ovulation in swine occurs 24 to 36 hours after the onset of estrus. This is similar to the 30 to 42 hour post onset of estrus period of ovulation reported by Oxenreider and Day (1965).

Fertilization rates of 95% or greater have been reported by Squiers et al. (1950), Self et al. (1955), Haines et al. (1959) and Perry and Rowlands (1962). Squiers et al. (1952) indicated that fertilization apparently is an "all or none" process. Warnick et al. (1951) observed that reproductive efficiency, as measured by ovulation rate and fertili-

zation rate increases with advancing sexual age, at least through three estrus cycles.

In a study of transport and cleavage of ova in swine, Oxenreider and Day (1965) reported that ova remained in the oviduct until 66 to 90 hours after onset of estrus. Among gilts slaughtered 66 to 75 hours post onset of estrus, 22 of 99 ova were recovered from the uterus. No ova were recovered from the uterus prior to 66 hours.

Data on the location and cleavage stages of recovered ova were reported. One- and two-cell ova were normally found in the oviduct 36-54 hours and 60-69 hours after the onset of estrus, respectively. Four-cell ova were usually found in the third and fourth quarter of the oviduct and in the first 5 inches of the uterine horn 60 to 90 hours post onset of estrus. Four- to eight-cell ova were recovered from the first 5 inches of the horn at 75-96 hours. Ova having more than eight blastomeres were not recovered until 78 hours, and these were generally recovered from the first 5 inches of the horn.

Generally, normal cleavage of ova is employed as the criteria for determining fertilization rate. Dziuk (1960), studying 31 unbred gilts, described ova fragments in 8 to 16 equal sizes that resembled normally-cleaving fertilized ova. Of 230 ova recovered from the uterine horns, 184 were fragmenting compared to only one fragmented ova out of 93 recovered from the oviduct. He concluded that there is a positive association between post-ovulatory age of ova and percentage of fragmented ova.

Hancock (1961) also reported spontaneous cleavage of unfertilized ova in unmated gilts. He suggested that this phenomena can be a considerable source of error when estimating fertilization rate, recommending that if cleavage is to be used as evidence of fertilization, 72 hours

after the onset of estrus is the most suitable time for recovery of ova. Examination of ova recovered subsequent to this time might indicate an apparent fertilization when in fact no fertilization had actually occurred.

CHAPTER III

MATERIALS AND METHODS

This study consisted of a single trial conducted at the Fort Reno Livestock Research Station from June 15, 1970, to November 16, 1970. A total of 60 Duroc X Beltsville first litter gilts at an average age and weight of 356 days and 304 pounds were used in this study.

Temperature Control

Temperature control was accomplished through the use of two temperature control chambers located inside a closed building at the Fort Reno Swine Production Unit. The design of these two chambers and details of the hot chamber, are illustrated in Figures 1 and 2, respectively.

The chambers measured approximately 12' x 12' x 8', with 5 inch double-wall insulation in both walls and ceiling. The chambers were equipped with slatted floors, instrument cages, and automatic watering systems.

Each chamber had two 22,500 BTU window-type air conditioners. The hot chamber was heated with a 50,000 BTU heater installed above the chamber. Thermostats capable of controlling temperature within a range of ± 2°F. of the desired temperature were located approximately 48 inches above the floor on the wall opposite the air conditioning units. It was not possible to regulate humidity in the two chambers. Readings ranged from 40% to 65% in the hot chamber and 58% to 70% relative humid-

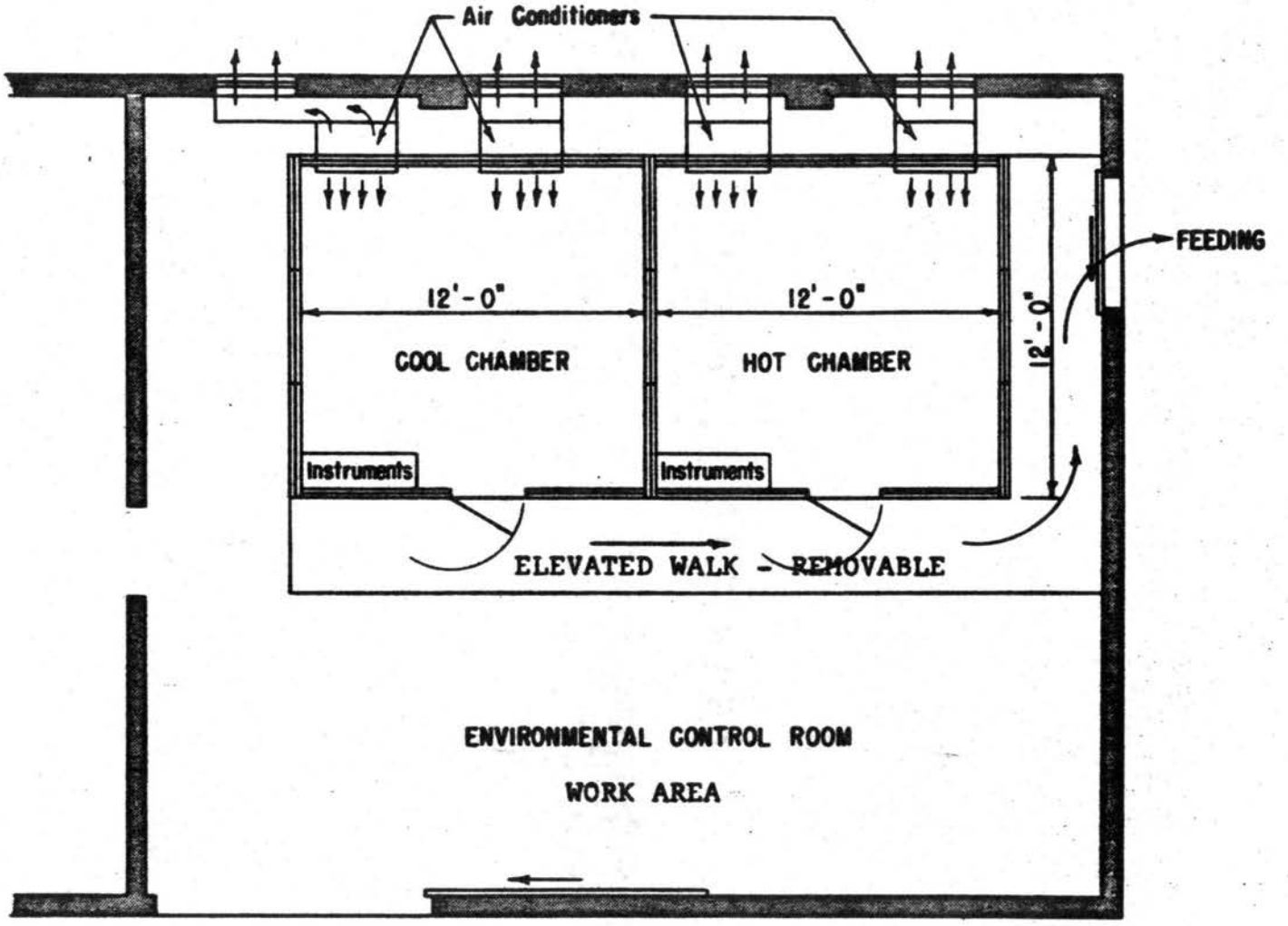

Figure 1. Environmental Control Room Showing Location of Chambers, Work Area, and Access Walk for Moving Gilts In and Out of Chambers

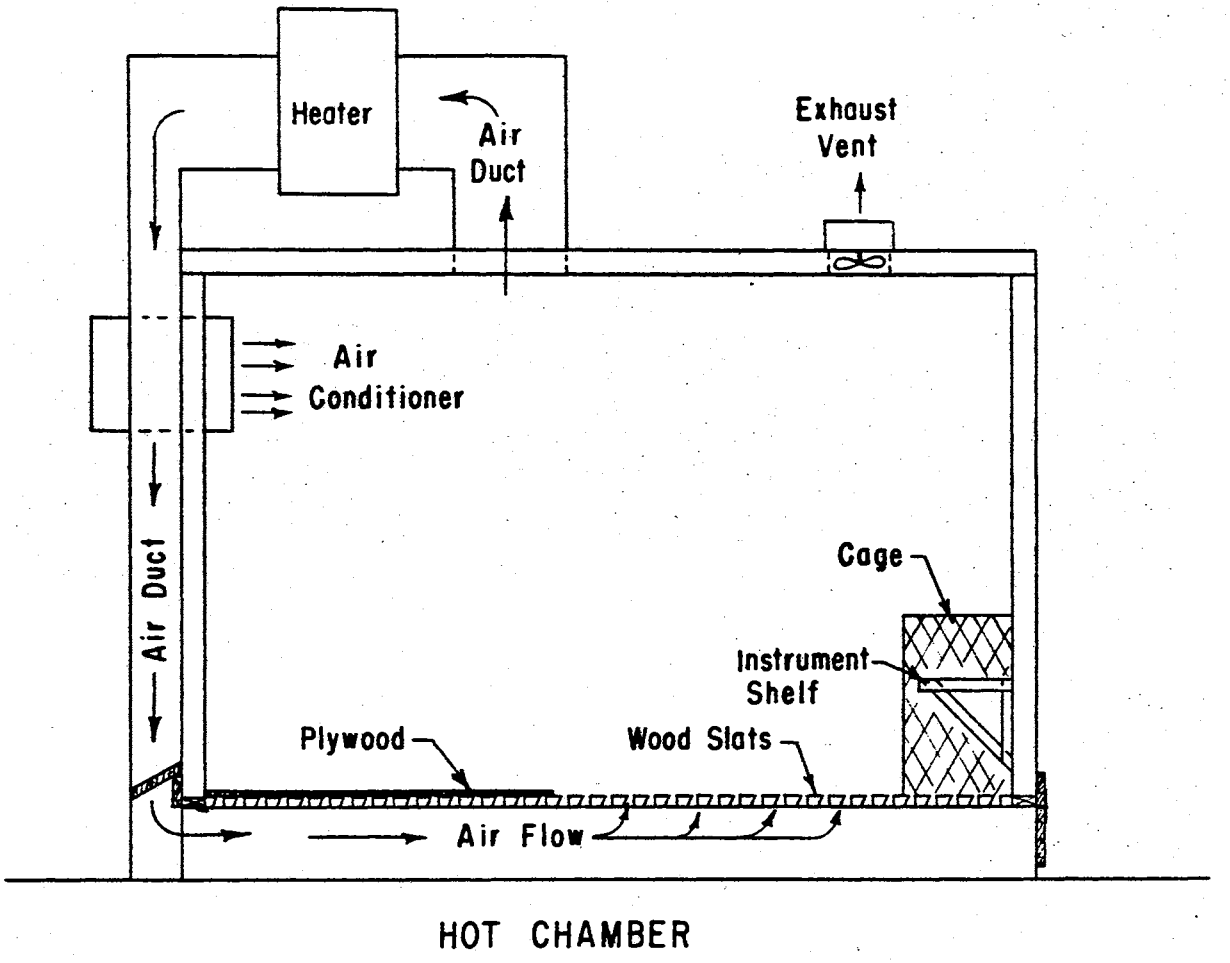

Figure 2. Cross Section of Hot Chamber Showing Location of Environmental Equipment

ity in the control chamber. A constant record of temperature and humidity was obtained by means of hygrothermographs located at floor level within the instrument cages in each chamber.

Artificial lighting was provided in each chamber for approximately 10 hours daily.

General Procedure

Gilts were observed through two complete estrous cycles prior to beginning treatment to establish an average cycle length for each gilt. Gilts were checked for estrus twice daily with vasectomized boars. When gilts had completed their second estrous cycle, they were randomly allotted to one of four treatment groups as indicated in Table I.

To provide a daytime stress period within the hot chamber, the thermostat was set to 96°F. at 7:30 a.m. and lowered to 90°F. at 4:00 p.m. The control chamber was maintained at a constant temperature of 74°F.

On day 15 of the third estrous cycle, gilts were placed in their allotted chambers. Gilts were removed from the chamber from 7 a.m. to 8 a.m. each morning and from 4 p.m. to 5 p.m. in the afternoon. During these periods, gilts were fed and checked for estrus. If in estrus, they were bred during these periods. Gilts were bred a maximum of four times, twice daily for each of the first two days in estrus. Fourteen yearling Hampshire boars of proven fertility were used in this study, with no boar mated to the same gilt more than one time.

Following breeding, gilts were returned to their allotted chamber until the end of estrus. Approximately 48 to 72 hours after their first breeding, treatment 1 gilts were moved into the control chamber and re-

TABLE I

TREATMENT SCHEDULE

Treatment Group	No. Gilts	Chamber Day 15 of estrous cycle to 48-72 hrs. post-breeding	At 48-72 hrs. post 1st breeding	At 22-28 days post 1st breeding
1	20	Hot	move into control chamber	slaughter for embryonic mortality
2	10	Hot	slaughter for ova recovery	
3	20	Control	leave in control chamber	slaughter for embryonic mortality
4	10	Control	slaughter for ova recovery	

mained there until 21 to 26 days following onset of estrus. Treatment 3 gilts were maintained continuously in the control chamber until 21 - 26 days post onset of estrus. Treatment 2 and 4 gilts were removed from the chambers 48 to 72 hours following first breeding for slaughter.

Feeding

The gilts were fed in individual stalls to insure uniform feed consumption. The ration fed during the study is given by Table II.

TABLE II

BREEDING HERD RATION

Ingredient	Amount, lb.
Wheat	994
Milo	500
Soybean Meal (44%)	260
Tankage	100
Alfalfa Pellets	100
Dicalcium Phosphate	20
Ground Limestone	6
TM Salt	10
Premix	10
TOTAL	2,000

All gilts were fed 2 pounds of the ration at each of the two daily feedings.

Water was available ad libitum from automatic watering devices inside the chambers.

Rectal Temperatures

Rectal temperatures were obtained from all gilts in the chambers at 7:00 a.m. and 1:00 p.m. by means of standard rectal thermometers. The morning reading was taken prior to elevation of the temperature in the hot chamber as an estimate of minimum daily rectal temperature for each gilt. The afternoon reading was made after 5 hours exposure to the elevated chamber temperature and, thus, at a time when rectal temperature should have been at the maximum.

Evaluation of Reproductive Tracts

All gilts in this study were slaughtered at Ralph's Packing Co., Perkins, Oklahoma, which is approximately 90 miles from Fort Reno. Slaughter was limited to three days during the week. Treatment 1 and 3 gilts were slaughtered between day 21 and 26 following first breeding for evaluation of embryo survival. Treatment 2 and 4 gilts were slaughtered between 53 and 70 hours following first breeding for recovery of ova.

Intact reproductive tracts were collected at time of evisceration, placed in plastic bags on ice, and taken to the physiology laboratory at Oklahoma State University.

Embryo Survival

Ovaries were cut free of the mesovarium and examined. Number of corpora lutea were recorded. Ovaries were then stored in a freezer until a later date at which time total corpora lutea from each ovary were excised and weighed on a Mettler scale.

The uterine horns were dissected longitudinally beginning at the cervical end and embryos removed for examination. Crown-rump measure-

ments were made on all embryos while enclosed in the amnionic sac. Those embryos that were obviously abnormal or degenerating, or those markedly smaller than was typical of that stage of gestation were classified as dead.

Ova Recovery

The reproductive tracts were placed on ice to minimize smooth-muscle contraction and possible displacement of ova. Recovery and examination of ova was accomplished 1 to 6 hours after recovery of the tract.

Number and condition of corpora lutea on all ovaries were noted. The oviduct was carefully trimmed free from the broad ligament to facilitate flushing. Approximately one-half inch posterior to the utero-tubal junction, the oviduct was separated from the uterus. A 10 guage needle was inserted into the fimbriated end of the oviduct and 10 cc of 0.9% saline solution flushed through the oviduct.

About one-half of the examinations were made using a technique whereby the flushed saline was collected directly on several microscope slides prior to examination under a stereoscopic microscope at 20X for presence and location of ova.

However, this technique was later modified so that one person could perform the flushing. Approximately 3 inches anterior to the utero-tubal junction, a cut was made leaving a segment of the horn attached to the oviduct. The uterine end was clamped tightly and 15 cc saline injected into the oviduct, through the utero-tubal junction and filling the uterine segment. The clamp was then slowly released above a watch glass, thus allowing the flushing fluid to be more precisely controlled and lessening the chance of losing ova. This procedure was repeated

four times for each oviduct.

The watch glass was transferred to the stage of a stereoscopic microscope and examined for presence of ova. An eye dropper was used for transferring ova from the fluid in the watch glass onto slides for detailed study at 200X and 430X using bright field microscopy.

If sperm were present in the zona pellucida and no gross abnormalities were noted, ova were considered fertilized. Ova showing shrunken cytoplasm, cytoplasmic vacuoles, ruptured vitelline membrane or zona pellucida, or cleavage with grossly unequal cell sizes were classified abnormal. If no sperm were evident in the zona pellucida of uncleaved ova, examination was made for patency of the cervix and uterine horns.

Statistical Analyses

Differences in number of embryos at 25 days post-breeding, embryo length, and treatment estrual cycle length were analyzed by analysis of covariance. The analyses of variance for these variables are given in Tables III and IV. These analysis were conducted according to Harvey (1960) and Graybill (1961).

All other response variables were subjected to an analysis of variance, as described by Snedecor and Cochran (1967), and tested by either the "t" test, or the "F" test, assuming common variance among stressed and control gilts.

TABLE III

ANALYSIS OF VARIANCE FOR NUMBER OF LIVE EMBRYOS AND AVERAGE EMBRYO LENGTH

Source	df
Total	36[2]
Covariable[1]	1
Treatment	1
Error	34

[1]Covariables were either number corpora lutea held constant for number of live embryo analysis, or days pregnant held constant for embryo length analysis.

[2]Three gilts did not furnish embryo data.

Two separate analyses described in Table III were made based on the following mathematical model given by,

$$Y_{ij} = \mu + \beta_1 (X_{ij} - \bar{X}..) + \tau_i + \varepsilon_{ij}$$

where,

Y_{ij} = individual observation of number of live embryos or average embryo length.

μ = mean number of live embryos or average embryo length.

β_1 = a regression coefficient for the effect of either number of corpora lutea or days pregnant, X_{ij} covariables, with $\bar{X}..$ being the overall mean associated with the appropriate covariable.

τ_i = an effect for the ith treatment (i_1 = Treatment 1, i_2 = Treatment 3).

ε_{ij} = the failure of the above model to estimate number of live embryos or average embryo length.

TABLE IV

ANALYSIS OF VARIANCE FOR TREATMENT ESTRUAL CYCLE LENGTH

Source	df
Total	58
Average Length of Pre-Treatment Cycle	1
Chamber	1
Disposal	1
Chamber x Disposal	1
Error[1]	54

[1]Error term used for test of significance.

The mathematical model for the analysis in Table IV is given by,

$$Y_{ij}K = \mu + \beta_1 (X_i - \bar{X}..) + C_j + D_k + (CD)_{jk} + \varepsilon_{ijk}$$

where,

Y_{ijk} = individual observation of treatment estrual cycle length.

μ = mean treatment estrual cycle length.

β_1 = a regression coefficient for the effect of pre-treatment estrual cycle length, X_i, a covariable, with $\bar{X}..$ being overall mean pre-treatment estrual cycle length.

C_j = an effect for the jth chamber (j_1 = hot chamber, j_2 = control chamber).

D_k = an effect for the kth disposal (k_1 = slaughter at 25 days, k_2 = slaughter at 2-3 days).

$(CD)_{jk}$ = an effect for the interaction of the jth chamber with the kth disposal.

ε_{ijk} = failure of the above model to estimate treatment estrual cycle length.

TABLE V

ANALYSIS OF VARIANCE FOR CORPORA LUTEA WEIGHT AND PERCENT EMBRYO SURVIVAL

Source	df
Total	36
Treatment	1
Error[1]	35

[1]Error term used to test treatment

TABLE VI

ANALYSIS OF VARIANCE FOR NUMBER OF CORPORA LUTEA

Source	df
Total	55
Disposal	1
Chamber	1
Disposal x Chamber	1
Error[1]	52

[1]Error term used to test disposal, chamber, and chamber x disposal.

The mathematical model for the analysis in Table VI is given by,

$$Y_{ij} = \mu + D_i + C_j + (DC)_{ij} + \varepsilon_{ij}$$

where,

Y_{ij} = individual observation of number of corpora lutea.

μ = mean number of corpora lutea.

D_i = an effect for ith disposal (i_1 = slaughter at 25 days, i_2 = slaughter at 2-3 days).

C_j = an effect for the jth chamber (j_1 = hot, j_2 = control).

$(DC)_{ii}$ = an effect for the interaction of the ith disposal with the jth chamber.

ε_{ij} = failure of above model to estimate number of corpora lutea.

CHAPTER IV

RESULTS AND DISCUSSION

General Comments

Of the 60 gilts used in this study, four were excluded from the analyses for various reasons. Three gilts assigned to Treatment 3 failed to conceive and were removed from the analyses of reproductive performance. One gilt allotted to Treatment 4 for ova recovery was excluded because of an abnormal reproductive tract that prevented sperm transport.

Of the three gilts failing to conceive in Treatment 3, two exhibited regressing corpora lutea at slaughter. The other gilt had corpora hemorrhagicum, demonstrating evidence of having recently ovulated. The reason for these failures to conceive was not readily apparent. However, two of the gilts lost 56 and 33 pounds during their 7 and 6 day confinement periods, respectively, prior to breeding. The third gilt lost 25 pounds. This extreme weight loss could be considered as a possible cause for failure to conceive, though confinement at 74°F. was not expected to cause such a dramatic weight loss.

All gilts exposed to heat stress prior to breeding were pregnant at slaughter. Exposure to heat stress did not markedly affect feed consumption in this study, as was previously reported, (Edwards et al., 1968).

Examination of the recovered ova from the left oviduct of the gilt

in Treatment 4 revealed all were of the one cell stage and no sperm were evident in the zona pellucida. It was discovered that the left uterine horn of her reproductive tract failed to communicate with the cervix. It is also interesting to note that this particular gilt was a control gilt that had failed to conceive at her first treatment estrus and was rebred to provide fertilization data. Nalbandov (1952) reported that this blind uterine horn disorder was observed in 5 out of 79 sterile female swine.

Rectal Temperatures - Prior to Breeding

Data from all gilts confined to the hot chamber prior to breeding were pooled and compared to the pooled data obtained from gilts confined to the control chamber for the same period. This data is presented graphically in Figure 3. The graph indicates that gilts showed a definite response to the elevated ambient temperatures during the first 2 to 3 days of confinement. Considering only hot chamber gilts, from day 4 onward afternoon rectal temperatures appeared to stabilized about $1^{o}F$. higher than morning rectal temperatures.

Rectal temperatures for hot chamber gilts averaged $102.5 \pm .12^{o}F$. which was significantly higher ($P < .001$) than the average of the control chamber gilts', $101.4 \pm .12^{o}F$. This agrees with the results of Omtvedt et al. (1971).

Control chamber gilts showed a fairly constant response to the $74^{o}F$. environment by stabilizing around $101.5^{o}F$. This temperature is somewhat below the considered normal of $102.5^{o}F$. quoted by Dukes (1955), but agrees with previous findings at this station, Edwards et al. (1968) and Omtvedt et al. (1971).

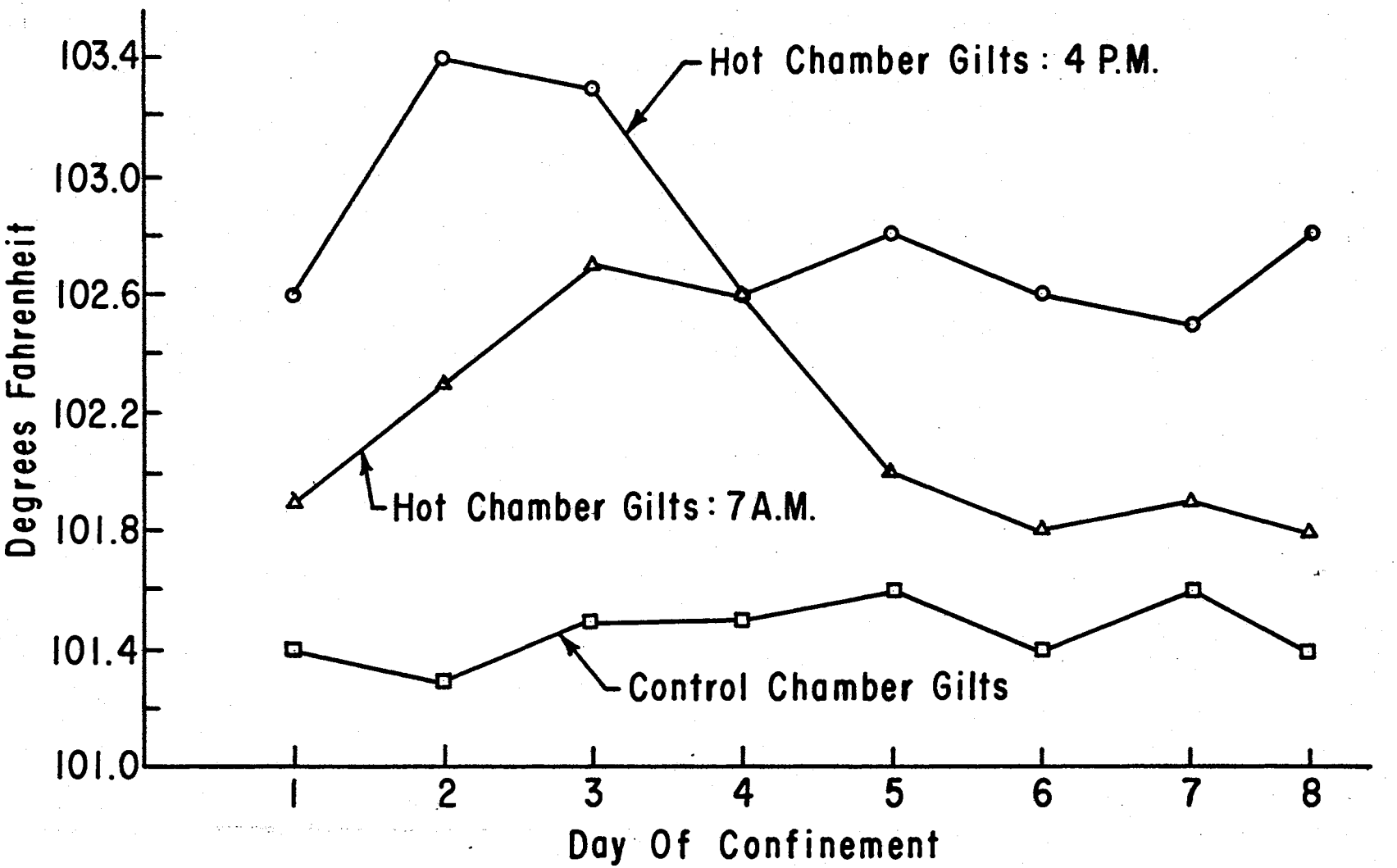

Figure 3. Rectal Temperatures of Gilts Confined to the Hot Chamber Prior to Breeding

Rectal Temperatures - Post Breeding

Rectal temperatures of gilts confined to the control chamber following breeding are presented in Figure 4. The average rectal temperatures of Treatment 1 gilts for this period were only slightly lower than that of Treatment 3 gilts, 100.98 ± .90 and 101.24 ± .90, respectively. This difference was not significant, however it does appear that the switch from the hot chamber to the control chamber may have caused the Treatment 1 gilts to over-adjust to the 74°F. environment. All temperatures averaged considerably lower than normal (102.5°F.; Dukes, 1955) for this period.

Ovulation Rate

Ovulation rates for all gilts confined to the hot chamber prior to breeding were not significantly lower than for the control chamber gilts. The results are presented in Table VIII.

TABLE VII

OVULATION RATE OF GILTS CONFINED TO HOT AND CONTROL CHAMBERS PRIOR TO BREEDING

Number of Gilts	Chamber	Number of Corpora Lutea
30	Hot	15.7 ± .56
26	Control	16.1 ± .60

Hot chamber gilts tended to have fewer corpora lutea than control gilts. A similar trend has been previously reported by Edwards *et al*.

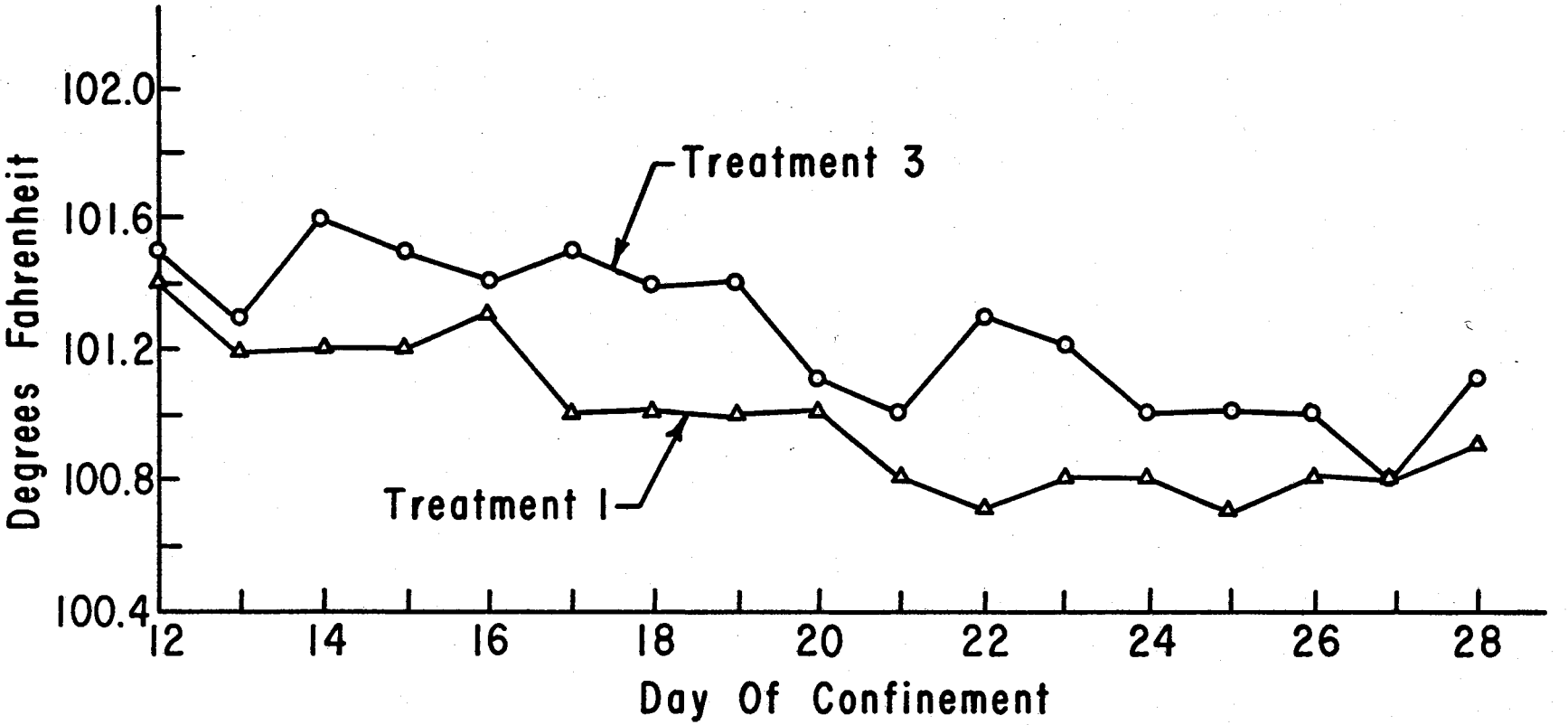

Figure 4. Average Daily Rectal Temperatures of Gilts Confined to the Control Chamber After Breeding

(1968) and d'Arce (1970) for a decreased number of corpora lutea following exposure to heat stress prior to estrus.

Ovulation rates for gilts slaughtered for ova recovery are presented in Table VIII. Hot chamber gilts ovulated more ova than control chamber gilts, however this difference was not significant.

TABLE VIII

MEANS AND STANDARD ERRORS FOR NUMBER OF CORPORA LUTEA AND PERCENT FERTILIZED OVA RECOVERED 2-3 DAYS POST-BREEDING FROM HEAT STRESSED AND CONTROL GILTS

Item	Treatment 2 Hot Chamber	Treatment 4 Control Chamber
Number Gilts	10	9
Number Corpora Lutea/Gilt	16.8 ± .97	15.3 ± 1.03
Percent Ova Recovered	70.2%	72.5%
Percent Fertilized of Recovered	85.24 ± 6.22	84.73 ± 6.22

Ovulation rates for gilts slaughtered for embryo survival determination showed a reverse trend, hot chamber gilts having fewer corpora lutea than control gilts, as shown in Table IX.

Fertilization Rate

To determine whether heat stress had an effect on fertilization or early cleavage, ova were recovered from heat-stressed and control-chamber gilts. Rate of fertilization was determined by percent of normal ova that had sperm in the zona pellucida.

Data on ovulation rate, percent ova recovery, and percent fertilized

TABLE IX

REPRODUCTIVE PERFORMANCE OF GILTS CONFINED TO ENVIRONMENTAL CHAMBERS PRIOR TO, DURING, AND POST-ESTRUS

Item	Treatment 1 Hot Chamber	Treatment 3 Control Chamber
Number gilts allotted	20	20
Number gilts pregnant at slaughter	20	17
Number of corpora lutea	15.1 ± .69	16.5 ± .75
Weight of corpora lutea, gm.	5.59 ± .27	6.11 ± .29
Total live embryos	226	216
Total dead embryos	4	5
Live embryos per gilt[1]	11.89 ± .68	12.01 ± .74
Embryo survival, percent	73.77 ± 4.2	77.09 ± 4.6
Embryo length, mm.[2]	15.88 ± .22	16.40 ± .24

[1]Adjusted number of embryos holding corpora lutea constant.

[2]Adjusted embryo length, holding days pregnant constant.

ova of those recovered are presented in Table VIII. No differences were observed between the two treatments. In fact, performance of the hot chamber gilts was slightly better than that of the control gilts. However, because of the low percentage of ova recovery and the limited number of observations, it is difficult to draw any conclusions from the data, other than there was no statistically significant difference between treatments as a result of pre-breeding exposure to heat stress.

It is quite possible that by allowing the heat-stressed gilts respite from high temperatures for the 16 hour period of only 90°F., the reproductive processes were not affected. Work by Thwaites (1969) and

Rich (1970) with sheep heat-stressed in a diurnally fluctuating environment supports this statement.

An interesting observation was made concerning the presence of "ghost cells" along with normal ova. A previous report by Squiers et al. (1952) described these as possibly fertilized ova but which had ruptured, allowing the cellular contents to escape, leaving only an empty cell wall. Two such cells, both recovered from heat stressed gilts, were noted in this study. While mechanical damage resulting from recovery cannot be ruled out as a possible cause of this event, it seems more plausible to regard it as a naturally occurring phenomena.

Embryo Survival and Development to 25 Days Post-Breeding

Reproductive performance of gilts slaughtered for determination of embryo survival is presented in Table IX.

In comparing the number of live embryos at 25 days post-breeding, no significant differences between treatments were detected when number of corpora lutea were held constant by use of covariance analysis. Therefore, the difference noted between live embryos is the result of difference in number of corpora lutea rather than the result of the treatment imposed.

Similarly, no differences in embryo length were detected when days pregnant were held constant. It would appear then, that exposure to high ambient temperature only prior to and during estrus has no effect on size of the embryo at slaughter. Average day of slaughter for hot gilts was 23.8 days post-breeding compared to 23.4 days for control gilts.

All comparisons presented in Table IX are not significant. A general trend is indicated however, in favor of the control-chamber gilts,

which ovulated more ova, had heavier corpora lutea, and possessed more and slightly larger embryos at slaughter. A similar trend to slightly larger embryos for control chamber gilts compared to gilts exposed to heat stress following breeding was reported by Edwards et al. (1968) and Omtvedt et al. (1970).

Length of Estrous Cycle

The average length of the estrous cycles for gilts prior to and during chamber confinement are summarized for each chamber in Table X.

TABLE X

AVERAGE LENGTH OF ESTROUS CYCLE PRIOR TO AND DURING CHAMBER CONFINEMENT

Comparison	Hot Chamber	Control Chamber
Number of gilts	30	29
Estrous cycle length prior to confinement, days	19.95 ± .26	20.43 ± .26
Estrous cycle length during confinement, days	20.28 ± .27	20.85 ± .27

Control chamber gilts exhibited a trend toward longer pre-treatment and treatment estrus cycle lengths than hot chamber gilts. However, these differences were not significant.

When length of cycle prior to confinement was held constant, no significant differences were found in the length of cycles during confinement between the two chambers.

Confinement to either chamber did not significantly lengthen treat-

ment estrus cycle length. Cycle length was not increased in gilts confined to the hot chamber, which conflicts with findings of Edwards et al. (1968), but is in agreement with d'Arce et al. (1970).

In summary, the effects of confinement and heat stress had no significant effect on estrual behavior among the gilts in this study.

CHAPTER V

SUMMARY

Two temperature control chambers were utilized to investigate the effect of heat stress on the reproductive performance of sexually-mature gilts. A single trial was conducted during the summer and fall of 1970, using 60 crossbred gilts. All gilts were confined to a hot or control chamber from day 15 of the estrous cycle to 48-72 hrs. post-breeding. Twenty gilts were slaughtered 53-70 hrs. post-breeding for ova recovery. Forty gilts were maintained in the control chamber from 48-72 hrs. post-breeding until slaughter at 21-26 days.

Gilts maintained in the heat chamber had significantly higher ($P < .001$) average daily rectal temperatures than gilts confined to the control chamber during the same period. Gilts demonstrated a definite response to increased temperatures during the first 2 to 3 days of exposure, then tended to adjust and stabilize at a lower temperature. Rectal temperatures from gilts confined to the control chambers following breeding were below that considered normal for swine.

There was no significant increase in length of estrous cycle during confinement for hot chamber gilts when compared to their average cycle length prior to confinement. Also, there was no significant difference in estrous cycle length during confinement between control and hot chamber gilts.

Differences in ovulation rate, fertilization rate, weight of corpora

lutea, number of viable embryos, and embryo length were not significant. However, heat stress gilts demonstrated slightly reduced reproductive performance as measured by these criteria.

The lack of significant differences between control and hot chamber gilts for various criteria observed seems to indicate that providing gilts a daily respite from high temperatures is sufficient to allow them to express normal reproductive performance.

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VITA

Stephen R. Payer

Candidate for the Degree of

Master of Science

Thesis: THE INFLUENCE OF HIGH AMBIENT TEMPERATURE PRIOR TO AND DURING ESTRUS UPON OVULATION RATE AND EMBRYO SURVIVAL IN GILTS

Major Field: Animal Science

Biographical:

Personal Data: Born in Riverside, California, September 9, 1947, the son of Mr. and Mrs. Steve Payer; married Kevyn d'Aulan Geyer March 21, 1970.

Education: Received the Bachelor of Science degree from California State Polytechnic College, Pomona, in May 1970, with a major in Animal Science.

Experience: Graduate Assistant at Oklahoma State University, 1969-1970.

Member: Alpha Zeta and Gamma Sigma Delta.