EFFECT OF FLUSHING AND EXOGENOUS GONADOTROPIN TREATMENT ON REPRODUCTIVE PERFORMANCE OF ESTRUS SYNCHRONIZED MATURE EWES

by

PETER C. HOPPE

B. S., California State Polytechnic College, 1964

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Animal Husbandry

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1966

Approved by:

Harold G. Spies
Major Professor

TABLE OF CONTENTS

INTRODUCTION

Synchronization of estrus in farm animals would facilitate the practice of artificial insemination. Advantages of estrus synchronization include increased labor efficiency during the breeding and parturition seasons and uniformity in age of descendants for marketing and replacement selection. Likewise, the application of estrus synchronization in reducing environmental variability in experiments is significant. The possibility of environmental interaction with stages of the estrous cycle could be eliminated in reproductive studies.

For sheep producers, increased ovulation rate and the subsequent elevation of progeny from the dam would be a financial asset. Hormone preparations such as human chorionic gonadotropin (HCG) and pregnant mare's serum (PMS) provide possible approaches for increased ovulation rates. Flushing, an increased nutritive intake with subsequent weight gain prior to breeding, has been shown to increase ovulation rate in the ewe. However, a high nutritive intake after breeding has been shown to have detrimental effect on embryo survival. Consequently, ewes which have been flushed prior to breeding must be placed on a lower nutritive intake after mating. More research must be conducted to determine the significance of flushing on subsequent ewe performance.

Estrous synchronization with oral progestogens is reported to bring a large percentage of ewes into estrus within a three day interval presumably by a mechanism of blocking the pituitary gonadotropins.

By contrast, flushing may act via the elevation of pituitary gonadotropins. PMS and HCG has been suggested to stimulate ovarian activity directly.

The present experiment was conducted to obtain preliminary evidence on whether these treatments would act synergistically, antigonistically or independently.

LITERATURE REVIEW

Flushing, the elevation of the nutritive state of the animal prior to breeding, has reportedly resulted in an increased lamb crop (Hulet et al., 1962; Clark, 1934; Foote et al., 1959; Campbell et al., 1959; El Sheikh et al., 1955) and litter size in swine (Robertson et al., 1951; Gossett et al., 1959; Haines et al., 1959; Self et al., 1955; Christian et al., 1952; Zimmerman et al., 1960). The influence of the nutritive level on reproductive performance in the bovine has been centered around the occurrence of the estrous cycle, the onset of post-partum estrus and fertility (Joubert, 1954; Bond et al., 1958; Wiltbank et al., 1957; Wiltbank et al., 1962; Wiltbank et al., 1964).

The effect of various feedstuffs on ovulation rate of the ewe has been studied; Hulet et al. (1962) conducted a three year study using 1200 Columbia and Targhee ewes. In this study ewes were supplemented with either 0.7 lb of oats or 1.0 lb of alfalfa pellets for 17 days prior to breeding and either 17 or 34 days post-breeding. Equivalent amounts of total digestible nutrients (TDN) were supplied to all ewes irrespective of oat or alfalfa supplementation. Oat supplementation increased the number of live lambs produced ($P > 0.10$) while alfalfa had no significant effect. Conclusively, oat supplementation increased reproductive performance even though equivalent amounts of TDN were present in both rations.

Darroch et al. (1950) increased the number of lambs born by supplementing the ewes with beet pulp pellets prior to and during the breeding period.

Although various feedstuffs have shown an effect on ovulation rate, energy levels per se have been studied extensively. Christian and Nofziger (1952) fed gilts a high and low plane of nutrition from 120 days of age until

farrowing. Ovulation rate was significantly ($P < 0.05$) higher in the high plane of nutrition (15.1 vs. 13.4 ova). Sorensen et al. (1961) also showed that high energy fed gilts shed more ova. Effect of energy intake on ovulation rate in gilts was also extensively studied by Zimmerman et al. (1960) by feeding a basal ration plus 1% glucose for two weeks prior to ovulation. Glucose feeding had a highly significant ($P < 0.01$) increase on ovulation (2.1 and 0.8 more ova in 2 experiments) and daily gain above controls. Gilts receiving a similar caloric intake furnished by lard (0.44%) were found to produce 1.9 more ova than basal fed gilts. Gilts fed lard (0.66%) at 150% the caloric intake of those fed glucose exceeded controls by 4.1 ova and the glucose and low fat gilts by 3.3 and 2.2 more ova, respectively.

The influence of energy level on the onset of postpartum estrus in the bovine was observed by Wiltbank et al. (1962). Mature cows were fed low and high levels of TDN (1/2 and required NRC levels) prior to calving. After calving, energy levels were either one-half or equal to NRC requirements for nursing cows. Cows were bred at the first postpartum estrus. Proportion of cows diagnosed pregnant after a 120 day breeding period for high-high, low-high, high-low and low-low energy levels was 95%, 95%, 77% and 20%, respectively. Conception rates were high for cows which received a high level of TDN after calving irrespective of the pre-calving energy level. However, detrimental results were observed when low levels of energy were fed after calving. Thus it appeared that low energy levels during the breeding season decreased the occurrence of estrus and fertility in the bovine.

Increased energy at calving or delayed until 28-35 days afterwards was compared with respect to reproductive performance (Wiltbank et al., 1964).

Three groups of cows were fed 75%, 100%, and 150% NRC recommended levels of TDN after calving. Two other groups were fed 50% TDN requirement the first

4-5 weeks after calving and then 100% or 150% TDN. Additional nutrients were supplied in excess of NRC requirements. The interval between calving and the first estrus was significantly ($P < .01$) shorter (49 days) in cows receiving 100% TDN after calving than those animals receiving 75% or 150% TDN. Feeding TDN levels above or below NRC requirements significantly ($P < .05$) delayed estrus by 73 and 72 days for 75% and 150% TDN, respectively. A significantly ($P < .05$) greater number (21%) of animals on the lower TDN level failed to exhibit estrus as compared to 7% and 8% of cows receiving 100% and 150% TDN, respectively. Delayed increase in TDN for 4-5 weeks post-partum also delayed onset of estrus but did not significantly affect the number of cows exhibiting estrus. Cows receiving 150% TDN had significantly ($P < .05$) greater follicular development prior to estrus than the 75% or 100% TDN groups. Fertility increased as the level of TDN increased with the 150% TDN group having the highest fertility of the three groups. Post-partum estrus was found not to be dependent on follicular development but large follicles may have conditioned the reproductive tract or ova in some manner to improve fertility (Wiltbank et al., 1964).

Further evidence that energy levels affect the estrous cycle was presented by Joubert (1954). Heifers were reared on a low and high plane of nutrition. No differences were observed in estrous cycle lengths; however, heifers reared on the low plane of energy exhibited anestrous during the winter until body weight was regained.

Bond et al. (1958) fed heifers either a low or high level of both TDN and digestible protein (DP). The heifers fed low TDN and DP lost weight (66 lb) over high TDN and DP fed heifers, but both groups of heifers continued to cycle during treatment. TDN levels were reduced in both groups on a comparable level while one group received a lower level of DP until the heifers ceased to

cycle. The cycling ceased at approximately the same time (133 days), but the heifers fed the low level of DP lost the greater amount of weight (23 lb). Thus the energy level may have an influence on the estrous cycle while the low DP levels tended to influence weight loss. Heifers were subsequently fed increased levels of TDN and DP until the estrous cycles were re-established. Heifers fed the low level of DP prior to cessation of the estrous cycle started cycling 49 days earlier with 98 lb less weight gain than the other group. A possible explanation for the low protein heifers cycling earlier is more responsiveness to an increased nutritive level.

Wiltbank et al. (1957) observed the effect of various levels of TDN and DP on the estrous cycle in Angus heifer calves. Fifty-four heifers were divided into nine lots and were fed for 250 days. Lots I, II and III were full-fed, lots IV, V and VI were 2/3 full fed and lots VII, VIII and IX received a ration which would maintain body weight. Lots I, IV and VII received 0.23 lb DP/cwt. Lots II, V and VIII received 0.15 lb DP/cwt; 0.06 lb DP/cwt was fed to lots III, VI and IX. Average daily gain, average number of days from the beginning of the experiment to first estrus and percentage of heifers showing estrus during the 250 day period for lots I through IX was as follows: 1.54 lb, 100 days, 100%; 1.38 lb, 82 days, 100%; 0.29 lb, 193 days, 67%; 0.77 lb, 148 days, 100%; 0.92 lb, 127 days, 100%; 0.30 lb, 203 days, 67%; 0.04 lb, 207 days, 50%; 0.15 lb, 186 days, 83% and 0.11 lb, 215 days, 33%; respectively.

These researchers have shown that a combination of TDN and DP are necessary for normal reproductive function. The low level of DP (0.06 lb/cwt.) and subsequent low energy reduced the number of heifers exhibiting estrus during the 250 day feeding period. Low protein in heifers full-fed, 2/3 full-fed and maintenance-ration fed reduced the number of heifers exhibiting estrus

by 33%, 50% and 70%, respectively. Similar effects were observed on the average number of days from beginning of the experiment to the first cycle. Although the energy and DP levels affected the percentage of heifers exhibiting estrus and the length of time from the beginning of the experiment to the first estrus, they did not affect the average length of estrus period (21.1 hours) in any lot. Consequently, normal reproductive function appears to depend on the availability of both energy and DP.

Foote et al. (1957) summed up the consequences of flushing indicating a difference in ovulation rate in ewes was not due to feeding but to the pre-flush weight and daily gain. Clark (1934) concluded a rising plane of nutrition could have a beneficial influence upon ewes in a relatively thin weight condition at the beginning of the feeding period. Zimmerman et al. (1960) found a correlation between daily feed consumption and increase in ovulation rate in gilts which approached significance (r- 0.32, P- 0.05-0.1).

A three year study was conducted by Self et al. (1960) to observe the effect of feeding three levels of nutritional intake on pasture to weanling pigs. An insignificant affect of growth rate on reproductive traits was observed in 36 litters studied. Foote et al. (1959) stated that the manner in which the ewe was fed during the early growing period was more important than nutritive state at breeding; however, Self et al. (1960) did not uncover a correlation between growth rate and reproductive performance in pigs.

Mature ewes have consistently been more responsive to flushing than yearlings or 2-year old animals (Hulet et al., 1962; Menzies and Banbury, 1963). The explanation for a greater response of mature ewes is not fully understood. Warnick et al. (1951) noted a steady rise in ovulation rate in swine with succeeding heat periods up to the fourth period. Fertility of the gilts also increased with advancing age. Pituitary weight and total gonadotropin potency

were increased in the older gilts. Consequently, mature ewes may respond to flushing over that of yearling ewes due to greater capacity for gonadotropin production and/or release.

The length of flushing for optimum ovulation rate at the time of breeding must be considered but the effect on embryo survival is equally important. Hulet et al. (1962) found no significant differences in lamb production when flushing was extended longer than 17 days before breeding. Extended feeding after conception may be detrimental to lamb production by increasing embryo mortality. A greater flushing effect on lamb crop was observed in ewes mated 13-18 days following the termination of feed treatment rather than those animals mated the first six days following the flushing period. Embryo survival may possibly have been increased by mating ewes 13-18 days after feeding compared with ewes mated immediately upon termination of feeding. El Sheikh et al. (1955) observed a lower embryo survival in ewes that were fed roughage plus grain after conception as compared to ewes receiving only roughage. Robertson et al. (1951) found a greater percentage of embryos present in low protein, limited fed gilts than full fed gilts.

Zimmerman et al. (1960) flushed gilts on the 8th, 12th and 16th day of the first cycle and slaughtered the gilts 3-5 days after the second estrus. Gilts flushed from the 12th and 8th day significantly ($P < 0.02$) increased ovulation rates over the controls while flushing from the 16th day approached significance ($P < 0.07$). Sorensen et al. (1961) fed gilts either a low or high energy intake from the time of weaning until the end of the first gestation period. High energy gilts shed more ova but the embryo survival was higher in the low energy fed gilts. Percentage of corpora lutea represented by embryos at 40 days after breeding was significantly ($P < 0.01$) increased in the low level of energy.

Christian and Nofziger (1952) either full fed 120 day old gilts or fed 70% full feed until after the first gestation. Live pigs farrowed was significantly ($P < 0.01$) increased in gilts fed a low plane of nutrition (7.4 vs. 4.7 pigs). Ray and McCarty (1965) fasted gilts for 0, 24, 48, and 72 hours after mating. Embryo survival was found to be higher in gilts which were fasted.

Thus it appeared that increased energy and protein levels were necessary for a flushing effect to occur and that the flushing period must exist for a definite period of time for a positive effect on ovulation rate. However, prolonged feeding of high energy rations resulted in an increase in embryo mortality with no additional increase in ovulation rate. A feeding period of approximately 3 weeks prior to breeding resulted in increased ovulation rate but embryo survival was not influenced unless feeding was continued after mating. However, embryo survival was found to increase when the gilt was fasted after mating but a nonsignificant decrease in ovulation rate was observed. Possibly exogenous gonadotropins could be administered as an agent for increasing ovulation rate and energy levels reduced for optimal maternal environment for embryo survival.

Bellows et al. (1963) studied the mechanism of nutritionally induced differences in ovarian activity in 128 mature white face ewes. Ewes were divided into two groups; one group received a maintenance ration of hay and the other received a gaining ration of hay plus grain in order to condition the animals prior to initiating the experiment. Sodium propionate was administered orally in a drench to one-half of the ewes in each of the two groups. The experiment was conducted during one estrous cycle after which the ewes were slaughtered. The sodium propionate did not increase ovarian follicular fluid, carcass weight or anterior pituitary weight. Grain supplementation

significantly increased follicular fluid ($P < .05$) and anterior pituitary weight ($P < .05$) as well as elevated carcass weight ($P < .01$).

The mechanism of flushing on increased ovulation rate may be proposed from the preceding evidence. The anterior pituitary weight increased with grain supplementation (Bellows et al., 1963). Howland et al. (1965) and Haines and Warnick (1959) observed an increase in pituitary weight in both ewes fed hay plus grain and full-fed gilts. An increase in anterior pituitary weight and in follicular fluid on the ovary indicates an increase in FSH release from the pituitary gland. Flushing may increase the gonadotropic content, FSH and LH, of the anterior pituitary by acting directly upon the gland or more plausibly by acting upon the releasing factors in the hypothalamus. Evidence that the nutritive level of the animal affects the pituitary content of tropic hormones is presented in the work of Meites and Fiel (1965). These observers concluded that starvation in the adult rat for 7 days reduced the hypothalamic content of SRF as well as the pituitary content of STH by 40-50% when compared with ad lib. fed rats. They further postulated that starvation decreased the synthesis and release of SRF from the hypothalamus and resulted in a decreased synthesis and release of STH from the pituitary gland. The mechanism of flushing may be one of increasing the synthesis and release of FSH-RF and LH-RF in the hypothalamus with a resulting increased release or synthesis of the gonadotropins from the anterior pituitary gland.

The effect of fasting on ovulation rate in the gilt was observed by Ray and McCarty (1965). Feed was removed at 0, 24, 48 and 72 hours after mating. Ovulation rate was slightly lower in gilts from which feed was withheld at breeding but the differences were not significant. Possibly fasting of the gilt prior to breeding decreased the available gonadotropic hormones which

resulted in a decreased follicular growth and ovulation. Future experiments in the realm of flushing may be more rewarding if the concern is gonadotropic hormone fluctuations in addition to ovulation rate of the animal on various nutritive planes.

Pregnant mare serum (PMS) has been shown to possess follicle stimulating with mild luteinizing properties (Lamond, 1964; Moore and Shelton, 1964). PMS rather than purified FSH has been used to stimulate follicular growth in farm animals due to the availability. Lamond (1964) reported PMS to have either predominantly follicle stimulating properties or ovulating action depending on the dosage and injection time. HCG (human chorionic gonadotropin) due to its LH like properties has been used in conjunction with PMS. Dziuk and Baker (1962) observed that HCG could cause ovulation in the gilt of all follicles on the ovaries resembling normal preovulatory follicles. HCG was ineffective, however, in causing ovulation in gilts who failed to develop follicles during the estrous cycle.

PMS has been used to attempt to (1) increase the number of follicles present on the ovary for ovulation (Gossett et al., 1963), (2) induce estrus in anestrous ewes (Lamond, 1964; Dutt, 1953) and (3) increase fertility in the ewe (Robinson, 1961; Lamond, 1964).

A single injection of PMS induced ovulation in the anestrous ewe but progesterone priming was essential for reasonable fertility (Lamond, 1964). A time dose relationship may exist between progesterone and PMS that could result in high fertility. Dutt (1953) observed that anestrous ewes injected subcutaneously with PMS (500 IU) ovulated but did not exhibit estrus without progesterone treatment. In fact many workers have suggested progesterone may be essential for inducing estrus in anestrous ewes. Lamond (1964) suggested administering PMS from 0 to 2 days after the withdrawal of progesterone.

The number of ewes lambing tended to be greater when PMS was given at the cessation of progesterone rather than 48 hr after hormone withdrawal. Lamond (1964) also noted a lengthening of estrus when PMS was given one day after cessation of progesterone. Duration of estrus was approximately 2-3 times the normal length in ewes reciving 3600 IU of PMS.

In an attempt to increase ovulation rate in the ewe, Neville and Neathery (1964) injected PMS, 750 and 1000 IU, on day 13 and 14 of the estrous cycle. PMS injections increased the lamb crop by 34% which was significantly ($P < .01$) higher than controls. The 1000 IU level was no more effective than 750 IU due to higher embryonic mortality in the former group.

As ovulation rate increased, reduced fertility was noted by Gossett et al. (1963). Ewes were injected with either 600, 800 or 1000 IU of PMS on day 14 of the estrous cycle. Average number of ova ovulated, as measured by corpora lutea counts, was 1.75, 2.25 and 5.0, respectively. The difference between 600 and 1000 IU was significant ($P < .05$). Although a large number of ova were ovulated with the higher doses of PMS, "embryo survival" was greatly reduced. Average number of ova fertilized for the 600, 800 and 1000 IU was 1.75, 1.50 and 1.57, respectively. Number of fertilized ova was determined by sacrificing ewes 48 hours after breeding and flushing the Fallopian tubes and uteri with physiological saline solution. Ova were examined under a microscope to determine if cleavage had taken place. In ewes allowed to lamb, conception rate and number of lambs born per ewe lambing for controls, 500, 800 and 1000 IU of PMS was 86.1%, 1.57; 67.4%, 1.69; 81.2%, 1.60 and 89.1%, 1.83, respectively. These workers and Hamond (1921) contend that the number of ova produced is a limiting factor in the fecundity of the ewe. Robinson (1951) stated that levels of PMS in excess of 1000 IU resulted in rapid ova transport down the Fallopian tube; consequently, as ovulation rate increased,

fertilization, recovery of ova and the proportion of cleaved ova were reduced.

The mechanism of action of PMS cannot be fully explained at this time. Wyss and Pincus (1964) found that PMS in the rat may influence the follicles by preventing some of them from undergoing atresia or adding a supplementary stimulus for ovulation. The response from a given dose of PMS has been shown to be affected by factors such as season of the year, age of the ewe and nutritive state and breed of ewes (Robinson, 1951).

HCG has been used to ovulate the gilt (Dziuk and Baker, 1962; Day et al. 1959) and ewe (Dziuk et al., 1964). Dziuk and Baker (1962) fed gilts 500 mg of MAP daily for 8, 9 or 10 days. HCG, 250 to 2000 IU per gilt, was injected subcutaneously, intramuscularly or intravenously on day 5, 6, 7, 8 or 12 after end of MAP treatment. Animals were sacrificed 40 to 72 hr after HCG injection. HCG treatment was found to ovulate all follicles resembling normal pre-ovulatory follicles. HCG was ineffective in causing ovulation in gilts who failed to develop follicles. All doses of HCG were effective in causing ovulation regardless of route of administration. Ovulation occurred about 48 hr after the HCG injection in the gilt.

HCG treatment has also been shown to cause ovulation in the ewe following MAP feeding (Dziuk et al., 1964). Ewes receiving 75 mg of MAP for 14 days did not ovulate prior to 74 hr post-feeding. HCG, 500 IU intramuscularly, 44 or 48 hr following MAP resulted in ovulation within 25 hr following the HCG injection.

Progestogens have not only been used for synchronization of estrus, but in combination with gonadotropins. They have been used also for induction of estrus in anestrous ewes (Cole et al., 1945; Dutt, 1953; Robinson, 1955). Wagner and Bush (1961) fed 5, 10, or 20 mg of CAP per day for 16 days to

anestrous ewes. Control ewes received 10 mg of progesterone daily. Treatment was followed with 1000 IU PMS on the 17th day. One-half of the ewes in each treatment were injected with 750 mcg of the Na-salt of estradiol 36 hr later. In a 2 day period, 6 of 7 ewes exhibited estrus in the 5 mg of CAP plus estradiol group compared to 12 of 13 ewes in the control group. The 5 mg group was the only group receiving CAP which exhibited estrus. A reduced ovarian response to PMS was observed in the CAP treated ewes.

Pursel and Graham (1962) found that estrus could be induced in anestrous ewes by pretreating the ewes with 10 mg of progesterone daily for 9-19 days followed with 20-50 mg of FSH 24-48 hr after end of progesterone. Estrus was induced in 91.3% of the ewes and 98.4% ovulated. FSH administered 24 hr after progesterone resulted in significantly ($P < .01$) greater variation in ovulation rate than when FSH was administered at 48 hr. Daily MAP, 10, 30, or 60 mg, for 12 days satisfactorily replaced injected progesterone. Progesterone pretreatment was necessary for inducing estrus since a single injection of 25 mg of FSH induced estrus in 17.6% of 17 treated anestrous ewes. Brunner et al. (1964) found that MAP alone did not induce estrus in anestrous ewes but 80% of the ewes exhibited estrus when PMS was administered.

Nellor (1960) reported the successful control of the time of estrus and ovulation in gilts by feeding MAP. Evan et al. (1962), Dziuk et al. (1964) and Hogue et al. (1962) found that the ewe responded quite satisfactorily to MAP while Hansel et al. (1961) reported successful synchronization of estrus in the bovine.

Hinds et al. (1964) conducted an experiment to determine the effectiveness of an orally-active progestogen (MAP) in controlling the time of estrus, time of lambing and to determine the minimal effective level for estrus control. Ewes were divided into several groups and received either 25, 50, 75

or 100 mg of MAP per ewe daily for 14 days. Ewes were fed individually or in groups to observe the effectiveness of synchronization. No relationship was observed between the level of MAP offered and proportion of ewes exhibiting estrus during treatment. Levels of 25, 50 or 75 mg of MAP were equally effective in inhibiting estrus. Ewes exhibiting estrus early in the treatment indicated a delay of effectiveness of MAP at the start of the treatment or ineffectiveness in preventing estrus during the late follicular phase of the estrous cycle. The levels of MAP from 11 to 75 mg per 100 lb of live body weight suppressed estrus and it may be important to consider the weight of the ewe when determining the daily minimal effective dose of MAP.

Eighty-two percent of the MAP treated ewes exhibited estrus during the first 5 days following cessation of MAP feeding. The highest number of ewes exhibiting estrus occurred on the third day (57%). The mean interval from last MAP feeding to estrus was 2.67, 2.95, 3.08 and 3.28 days for 25, 50, 75 and 100 mg of MAP per ewe per day, respectively. A direct relationship between level of MAP and interval from last feeding to estrus was evident. This observation was supported by Wagner and Bush (1961) as well as Evans *et al.* (1962).

There was little difference in the percentage of ewes lambing in all groups except for the 25 mg dose. The 25 mg dose suppressed estrus but had an adverse affect on number of ewes lambing. Evans *et al.* (1962) found that ewes on a 90 mg dosage of MAP had the lowest variance for post-treatment estrus and the highest fertility as measured by lambing rates. Ewes receiving large levels of progesterone have shown higher ovulation rates due to number of follicles available for ovulation (Evans *et al.*, 1962; Foote *et al.*, 1958). Significantly ($P < .01$) more control than treated ewes (72% vs 59%, respectively) lambed to the first service. Birth weight and sex ratio of lambs were not

influenced by MAP treatment. The synchronization of lambing indicates that group feeding was as effective as individual feeding.

Dziuk et al. (1964) studies with progestational compounds have been oriented towards obtaining a dependable method for ovulation control in ewes. In one experiment, cycling ewes were administered 60 mg of MAP per ewe daily with a balling gun. Treatment began on different days of the cycle and ended when the ewes were killed 17 or 22 days after the last heat.

Ewes which were killed 22 days after last heat, had significantly ($P < .05$) larger follicles than ewes killed 17 days after last heat. Evidence reported by other workers also indicates that ovarian follicles continue to grow during progesterone treatment (Foote et al., 1958 and Lamond, 1964). Extremely large levels of progesterone, 100 mg daily for 35 days resulted in depressed follicular size and total weight of follicular fluid (Labhsetwar et al., 1964). Five hundred milligrams of MAP administered to gilts for 9 days completely inhibited follicular growth (Dziuk and Baker, 1961).

In a second experiment by Dziuk et al. (1964), ewes received either 40 or 60 mg of MAP per ewe daily for 14 days via a balling gun. Ewes were killed 24 to 96 hr after last MAP treatment to determine interval from last MAP feeding to onset of estrus and ovulation. Levels of MAP inhibited estrus and ovulation in most ewes. Six ewes were marked in experiment II during treatment. These ewes were killed on the day they were marked and no large follicles or ovulations were observed on the ovaries. Follicular growth increased rapidly between 24 and 48 hr after treatment. Follicles achieved maximum size at least 24 hr before expected ovulation as no difference in mean follicle size was detected between 48 and 96 hr after treatment. Both 40 and 60 mg of MAP daily resulted in no exhibition of estrus until 48 hr after last treatment. Of the ewes exhibiting estrus following treatment, 43% of the ewes

showed heat between 54 and 60 hr after last MAP feeding.

Hogue et al. (1961) administered 60 mg of MAP per ewe per day for 20 days. Of the ewes exhibiting estrus, 92.5% were observed in estrus 50-64 hr after last MAP feeding. Comb et al. (1961) fed 120 mg of MAP per head per day and observed 92.6% of the ewes in estrus on day 3, 4 and 5 after cessation of MAP. Evans et al. (1962) reported that 89.3% of ewes receiving 50 or 60 mg of MAP daily exhibited estrus before 8 days following treatment. In the bovine, Nelms and Combs (1961) have shown that cows receiving 220 mg of MAP per day for 15 days exhibited estrus on day 2 and 3 after the last feeding. Of 10 cows fed 0.8 mg of MAP per lb of body weight for 15 days, 9 of these cows came in heat on day 3, 4 and 5 following withdrawal of the hormone. Hinds et al. (1964) stated that estrus occurred 3 days post-treatment for 60 mg of MAP per ewe per day but the interval was dependent on dosage. Interval from end of MAP treatment to onset of estrus increased as the length of treatment increased (Evans et al., 1962; Dutt, 1953). Interval was longer for 18 day than for a 15 day treatment. Dziuk et al. (1964) observed no correlation between last MAP treatment at onset of estrus or ovulation. Lack of a correlation between onset of estrus and ovulation may account for low fertility following estrus synchronization.

Hogue et al. (1961) conducted an experiment to study the effect of natural and artificial breeding on MAP treated ewes. Group 1 cycled naturally and bred naturally, group 2 cycled naturally and bred artificially while group 3 received 60 mg of MAP per day for 20 days and one-half (20 ewes) were bred naturally and the other half artificially. Conception rates for group 1 and group 2 were 50% and 49%, respectively. Of the ewes fed MAP, conception rate was 61% for those bred naturally while only 16% conceived by artificial insemination. Robinson (1961) observed that ova shed following progesterone

treatment in the ewe appeared to be normally fertilizable. The time of ovulation relative to onset of estrus appeared to fall within normal limits (12-24 hr after estrus). Results suggested that progesterone treatment caused no gross endocrine imbalance at the time of ovulation and fertilization. Spread of ovulation was too great to permit satisfactory conception rates to a single insemination and a satisfactory practice was to inseminate 3 times at 12 hr intervals.

Conception rates in artificially inseminated cows are considerably higher than in other farm animals. Possibly efficient technique accounted for increased conception. Nelm and Combs (1961) synchronized estrus in 2 year old heifers by feeding 250 mg of MAP daily for 14 days. Heifers were inseminated on day 3, 4 or 5 following treatment. In treated heifers, 40% became pregnant to one service. Control heifers were inseminated over a 21 day period with 60% conceiving.

Darwash et al. (1965) fed melengesterol acetate (MGA) to dairy cows for 18 days starting on day 7 or 32 postpartum. MGA, at a level of 0.4 mg per day, was found to be about the minimal dose for estrus synchronization. Conception rates indicated no effects due to MGA treatment. Of treated cows, 78% conceived to an average of 1.8 services per conception as compared to 82% of the controls conceiving to 2.06 services. Seven of 49 MGA treated cows developed follicular cysts and failed to ovulate.

Conception rates in swine treated with oral progestogen compounds have been quite low and the presence of cystic follicles have accounted for the majority of failures (Hansel, 1964).

Three other compounds used for estrus synchronization in swine other than MAP are CAP, ICI and AMP. Wagner and Seerley (1961) fed various levels of CAP for 18 days to gilts. Estrus periods were inhibited by 16.5 mg per day

and follicular development was inhibited at the 25, 32 and 50 mg dosage. Gilts exhibited estrus 3-7 days after CAP withdrawal in the 25 or 32 mg level. Of the treated gilts, 20% of the gilts had cystic follicles 5 days after withdrawal of CAP. Most of the gilts with cystic follicles were among the higher treatments.

Pond et al. (1965) fed gilts 0.66 or 1.1 mg of 17a-acetoxy-6-methypregnan-4, 6 dien-3, 20 dione (AMP) per kg of body weight daily for 15 days. Levels of AMP were 100% effective in inhibiting estrus, but cystic follicles were found in 4 of 5 gilts fed low level and 1 of 5 fed high levels. In a second experiment, Pond et al. (1965) fed 16 mg of 3-methyl ether of ethynyl estradiol (MEE) daily for 9 days followed by 1.32 mg of MAP per kg of body weight for 9 days. Gilts were naturally mated at the first estrus. The treatment inhibited estrus in 29 of 31 gilts and conception rates were 81.8% for controls and 77.8% for treated gilts. Oral administration of the estrogen derivative followed by progesterone derivative resulted in minimum cystic follicles and provided a practical means of synchronizing the estrous cycle of gilts with a high level of fertility.

A relatively new compound, dithio-carbamoylhydrazine (ICI-33,828), has shown fairly good results in synchronization of estrus in the gilt. Stratman and First (1965) fed 0, 58, 116 and 232 mg of ICI for 16 days starting on day 3, 13 or 18 of the estrous cycle. No significant difference among treatment groups in corpora lutea, litter size, percent conception at 3 and 25 days gestation and 25 day embryo survival. Longer intervals occurred from withdrawal to estrus as dosage level increased.

Gerrits and Johnson (1965) fed 0.90-1.06 mg per kg of body weight from day 1-17 of the cycle to one group of gilts and 1.39-2.14 mg per kg of body weight for 19 days. All gilts expressed estrus 5-8 days after removal of the

hormone. Conception rate based on non-return estrus at 25 days for controls, 0.90-1.06 mg and 1.39-2.14 mg was 80%, 80% and 81.8%, respectively. Neither embryo survival or litter size was significantly different from controls. Thus ICI may be the answer to estrus synchronization of gilts but further trials need to be conducted to confirm the previous work.

METHODS AND MATERIALS

Forty-eight mature cycling whiteface western ewes were weighed and randomly divided into two lots of 24 animals each. Vasectomized rams with paint-marked briskets were run with each lot of ewes for detection of estrus prior to and throughout the treatment period. All ewes used in the experiment had cycled at least twice before they were assigned to the experiment. Lot I received a maintenance ration, as recommended by NRC, while lot II received a high-concentrate ration for a 17 day period. Day 0 was designated as the first day of feeding (Fig. 1).

Table 1 presents the ingredients and calculated nutritive value for the maintenance and high-concentrate rations (Morrison, 1959). The maintenance ration contained 1.39 lb of TDN in the daily allotment of 2.5 lb total feed. The ration was formulated by using 95% dehydrated alfalfa and 5% sorghum grain. The 4.0 lb of allotted high-concentrate ration fed daily contained 60% dehydrated alfalfa and 40% sorghum grain with 2.58 lb of TDN. Protein content of the maintenance and high-concentrate rations was 0.31 lb and 0.44 lb in the daily allotted feed, respectively. Both rations were pelleted and ewes in both lots were group fed twice daily.

Repromix, MAP (6-methyl-17-acetoxy progesterone) in soybean oil meal, was added to both rations before pelleting in order that 60 mg of MAP was present in the daily consumption of feed.

Fig. 1. Scheme of treatments.

Ewes receiving the high-concentrate ration developed scours and failed to consume their feed about 3 days after starting the test. Daily consumption of the pelleted ration was reduced to 3.4 lb and ewes were given an additional 0.5 lb of alfalfa hay. Ewes continued to receive 60 mg MAP daily via adjusting the repromix level in the amount of pelleted ration consumed.

On day 15, 12 ewes, one-half the total number, from each lot were injected subcutaneously with 750 IU PMS (pregnant mare's serum). MAP treatment was discontinued on day 17 and ewes were weighed 12 hours after the AM feeding. Twelve ewes, 6 PMS treated and 6 non-treated, in both the flushed and non-flushed lot were injected intramuscularly with 500 IU HCG (human chorionic gonadotropin) on day 19.

Ewes exhibiting estrus after MAP treatment were hand mated to different fertile rams at 12 hour intervals throughout estrus. Ewes not exhibiting estrus were laparotomized 48-72 hours after detection of the subsequent estrus period to determine ovulation rate at the cessation of MAP treatment. Ewes returning to estrus were not rebred and were eliminated from the experiment.

Twenty-six days post-breeding, non-return ewes were laparotomized to verify pregnancy and count corpora lutea. Ewes were carried in dry lot during gestation and the number of ewes lambing and number of lambs born was observed. Data were analyzed by analysis of variance of factorially arranged treatments as described by Snedecor (1964).

RESULTS

Average weight gain per ewe (table 1a) for the 24 flushed ewes was 7.1 lb while non-flushed ewes lost an average of 3.1 lb over the 17 day period. This difference was highly significant ($P < 0.01$). PMS treatment depressed weight gain by an average of 2.3 lb per ewe in both flushed and nonflushed groups

and the difference approached significance ($P < 0.08$). Weight of ewes was not affected by HCG treatment since it was administered 36 hours after ewes were removed from the flushing ration.

No ewes were marked by the raddled vasectomized rams during the 17 day period of 60 mg MAP per ewe daily. Twenty-three of the 24 flushed ewes were observed in estrus at the end of MAP feeding (table 2). Thirty-five percent were observed in estrus by 36 hours after cessation of MAP treatment while 52% were in estrus between 36 and 48 hours. The remaining 13% of the flushed ewes exhibiting estrus were observed between 48 and 84 hours following MAP treatment. Eighteen of the 24 ewes fed a maintenance ration exhibited estrus following MAP. Of these 18 ewes, 28% were in estrus by 36 hours while 67% and 5% of the ewes exhibited estrus by 36 to 48 and 48 to 84 hours, respectively. Consequently, 23 (96%) of the flushed ewes exhibited estrus within 84 hours post-MAP treatment while only 18 (75%) of the ewes fed the maintenance ration were observed in estrus. Flushing, irrespective of other treatments, significantly ($P < 0.05$) increased the number of ewes exhibiting estrus but had no apparent effect on the time from MAP withdrawal to estrus.

HCG (human chorionic gonadotropin) increased ovulation rate by an average of 0.5 ova per ewe above that of other treatments (table 3). PMS (pregnant mare's serum) treatment tended to have a detrimental effect by depressing the ovulation rate per ewe by an average of 0.5 ova. However, neither the effect of HCG or PMS was statistically significant at $P < 0.05$. HCG in combination with PMS increased the ovulation rate only slightly compared to the ovulation rate of nontreated ewes. This may be the result of the detrimental effect of the PMS on ovulation rate. Six flushed ewes ovulated 0.5 ova above six non-flushed ewes, but this difference was not significant ($P > 0.10$). Flushing in combination with PMS or HCG treatment

did not significantly increase ovulation rate.

Of 41 ewes exhibiting estrus and subsequently being bred, 18 (44%) of these ewes conceived to the first mating as evidenced by a functionally appearing corpus luteum at laparotomy 26 days post-breeding. Eleven (63%) of these 18 ewes produced one or more live lambs (table 4). Flushing, irrespective of the other treatments, increased the number of ewes lambing by 82%, but the difference was not significant ($P > 0.10$). PMS treatment reduced the number of ewes lambing by 46% which approached significance ($P < 0.10$). HCG treatment decreased the number of ewes lambing by 28%. PMS in combination with HCG resulted in complete abolishment of conception in 12 ewes thus treated. Consequently, flushing increased the number of ewes lambing but PMS, HCG and the combination of PMS and HCG had a detrimental effect on lambing.

Fifteen lambs (table 4) were born from the 11 ewes lambing which is an average of 1.4 lambs per ewe. Number of ewes lambing were too few for valid conclusions concerning treatment effects on lambing rate.

Table 1. Nutrient content of rations used for maintenance and flushing.

		DM lbs.	DP lbs.	TDN lbs.
Maintenance ration				
2.38 lbs.	Dehydrated alfalfa meal	2.21	0.30	1.29
0.12 lbs.	Sorghum grain	0.11	0.01	0.10
2.50 lbs.		2.32	0.31	1.39
Flushing ration				
2.40 lbs.	Dehydrated alfalfa meal	2.22	0.31	1.30
1.60 lbs.	Sorghum grain	1.45	0.13	1.28
4.00 lbs.		3.67	0.44	2.58

Table 1a. Weight change of flushed and non-flushed mature whiteface western ewes.

Treatment	Number of ewes	Wt. change ave. (lb)
Flushed ewes	6	+6.7
Flushed and PMS day 15	6	+6.4
Flushed and HCG day 19[a]	6	+10.0
Flushed and PMS day 15 HCG day 19	6	+5.4
Average		7.1*
Non-flushed	6	-1.8
Non-flushed and PMS day 15	6	-3.8
Non-flushed and HCG day 19	6	-2.2
Non-flushed and PMS day 15 HCG day 19	6	-4.4
Average		-3.1

[a]HCG injections were administered after ewes had been weighed.

*Significantly ($P < 0.01$) different from non-flushed group.

Table 2. Number of ewes exhibiting estrus following MAP treatment.

Treatment	No. ewes	No. ewes exhibiting estrus	No. ewes exhibiting estrus by various time intervals following MAP treatment		
			48 hr.	60 hr.	84 hr.
Flushed	6	6		5	1
Flushed plus PMS day 15	6	6	3	3	
Flushed plus HCG day 17	6	5	3	1	1
Flushed plus PMS day 15 HCG day 19	6	6	2	3	1
		23*	8 (35%)	12 (52%)	3 (13%)
Non-flushed	6	6	3	3	
Non-flushed plus PMS day 15	6	4	1	3	
Non-flushed plus HCG day 19	6	4		3	1
Non-flushed plus PMS day 15 HCG day 19	6	4	1	3	
		18	5 (28%)	12 (67%)	1 (5%)

*Significantly ($P < 0.05$) different from non-flushed ewes.

Table 3. Ovulation rate of flushed and non-flushed ewes.

	Ave. ov.
Flushed	1.7
Flushed plus PMS day 15	1.3*
Flushed plus HCG day 19	2.3*
Flushed plus PMS day 15 HCG day 19	1.3
Non-flushed	1.2
Non-flushed plus PMS day 15	1.2*
Non-flushed plus HCG day 19	2.3*
Non-flushed PMS day 15 HCG day 19	1.5

*Differences between groups approached significance ($P < .07$).

Table 4. Number of lambs born of ewes which lambed.

Treatment	No. ewes lambing	Number of lambs born
Flushed	4	4
Flushed plus PMS day 15	3	4
Flushed plus HCG day 19	3	6
Flushed plus PMS day 15 HCG day 19	0	
Total	10*	14
Non-flushed	0	
Non-flushed plus PMS day 15	0	
Non-flushed plus HCG day 19	1	1
Non-flushed plus PMS day 15 HCG day 19	0	
Total	1	1

*Difference flushed and non-flushed ewes approached significance ($P < .09$).

Table 5. Analysis of variance of experimental data [F(1,35).05 = 4.12].

Weight change (Table 1a)

	MS
F	72.92* +
P(MS)	3.57**-
H(CG)	0.08
F,P	0.02
F,H	0.49
P,H	0.96
F,P,H	0.71

* F(1,40) 0.01 = 7.31
**F(1,40) 0.08 = 3.34

Ewes exhibiting estrus (Table 2)

	MS
F	4.33*+
P(MS)	0.17
H(CG)	1.58
F,P	1.58
F,H	0.17
P,H	1.58
F,P,H	0.17

* F(1,35) 0.05 = 4.12

Ovulation rate (Table 3)

	MS
F	0.20
P(MS)	3.74* -
H(CG)	3.74* +
F,P	0.20
F,H	0.55
P,H	1.80
F,P,H	0.02

* F(1,35) p.07 = 3.58

Ewes lambing of ewes bred (Table 4)

	MS
F	3.31*+
P(MS)	3.24*-
H(CG)	0.71
F,P	0.53
F,H	1.53
P,H	2.12
F,P,H	1.06

* F(1,34) 0.10 = 2.88

DISCUSSION

Little evidence exists to explain the mechanism by which flushing increases ovulation rate. Possible hypothesis are; (1) elevation of releasing factors from the hypothalamus (2) direct effect on the gonadotropin output from the anterior pituitary gland (3) or increased ovarian sensitivity to endogenous hormone. Mulinos and Pomerantz (1940) reported a decrease in anterior pituitary activity with reduced caloric intake. Body weight gain and skeletal growth are also reduced (Ershoff, 1952). Meites and Fiel (1965) indicated that the hypothalamus of the rat was affected by starvation and that the anterior pituitary was thus indirectly affected by caloric intake. A decrease in SRF (somatotropic releasing factor) content in the hypothalamus of starved rats resulted in a subsequent decrease in anterior pituitary content of STH (somatotropic hormone). The possibility exists that a reverse effect on the releasing factors may be observed with elevated caloric intake. This may occur either via increased synthesis or release of the hypothalamic factors.

Gonadotropin potency in the pituitary of the ewe, both FSH and LH, increased with an elevated caloric intake (Bellows et al., 1963). Pituitary gonadotropin concentration increased only slightly but an increase in pituitary weight resulted in greater hormone potency. The preceding evidence may indicate that flushing stimulated hypothalamic activity with a subsequent elevated releasing factor output to the anterior pituitary. Although insufficient literature exists to choose a logical pathway, releasing factors may influence the anterior pituitary by three means: (1) cell proliferation, (2) stimulation in hormone synthesis (3) and release of synthesized hormones. Consequently increase in anterior pituitary weight may be due to increased

number of cells, which results in elevated potency of the gland, but the concentration of the gland may not be affected since individual cells may be producing similar amounts of hormone. It would be of interest to observe the maximal hormone output of an individual cell incubated in vitro with the appropriate releasing factor for a given period of time. Subsequent manipulation of the nutritional status of the cell might provide evidence of its sensitivity to the releasing factor. Thus the effect of caloric intake on anterior pituitary activity may be due to increased cell sensitivity and/or increased elaboration of releasing factor.

Ovarian sensitivity does not seem to be significantly increased with elevated caloric intake. Bellows et al. (1963) reported no increase in ovarian sensitivity to exogenous PMS in the ewe with flushing. Therefore, the available evidence indicates that the mechanism of flushing influences neurohumoral factors rather than end organ sensitivity. Greater quantity of releasing factors may result in cell proliferation of the anterior pituitary and thus increased potential of the pituitary to synthesis and release of gonadotropins.

Literature indicates that PMS possesses mainly FSH-like activity (Cole and Hort, 1930) and thus amy provide an additive effect to flushing. Although ovarian sensitivity does not increase with flushing, the ovary appears to remain sensitive to endogenous as well as exogenous hormones (Bellows et al., 1963). Time of administration of PMS during the estrous cycle or with oral progestogen treatment may be critical for normal reproductive function (Lamond, 1964).

A highly significant correlation between gonadotropic hormone content of the pituitary gland, follicular growth and day of cycle has been reported in the sow (Robinson and Nalbandov, 1951). Day 8 of the cycle was characterized

by a sharp increase in pituitary gonadotropin content which remained high until the end of the estrous cycle. Maximum follicular numbers were observed at day 13 and 20 with change in follicular size occurring at day 14 and continuing to the second day of heat. A drop in follicular number was observed between day 20 and first day of heat and may be attributed to decreased FSH release from the pituitary. Similar changes in gonadotropin content of the pituitary gland during the estrous cycle in swine has been reported (Parlow et al., 1964). The ratio of FSH and LH remained constant during the estrous cycle. The most beneficial time of PMS administration for added follicular growth appears to be near the onset of estrus. This period of time is normally characterized by a decrease in follicular number probably due to decreased FSH release. Since PMS possesses FSH-like activity (Cole and Hart, 1930), PMS administration immediately prior to or at the onset of estrus may prevent the subsequent follicular atresia. Possible evidence for this fact was reported by Wyss and Pincus (1964) in that PMS has an effect on preventing follicular atresia in the rat.

PMS treatment may also have an additional influence on ovulation by stimulating LH release from the pituitary gland. Quinn and Zarrow (1964) reported that PMS injection in the rat was followed by hypothalamic stimulation and subsequent release of LH 56 hours after injection. Whether PMS affects the hypothalamus in other species needs to be determined.

In summary of PMS treatment, a hypothetical case may be proposed for the sow since the anterior pituitary gonadotropin levels have been determined. The sow has a normal estrous cycle length of 21 days and ovulation normally occurs on the second day of estrus. If PMS was administered on day 20, the time at which follicular atresia occurs, PMS may provide follicular activity

to maintain follicles which would normally regress. If PMS influences the hypothalamus of the sow by causing a release of LH 56 hours post-injection, an additional surge of LH from the action of PMS may enhance ovulation rate. PMS may thus prevent follicular atresia and in addition provide an added amount of LH for increased ovulation rate. The optimal time of PMS administration to other species might be determined subsequent to knowledge of estrous cycle changes in gonadotropin levels.

HCG administration may increase ovulation rate by augmenting endogenous LH levels. Present literature suggests HCG has ovulatory activity, but little follicle-stimulating properties (Zarrow et al., 1964). Following HCG, follicles can be ruptured that may not normally ovulate, but HCG does not ovulate follicles unless they resemble normal preovulatory structures (Dziuk and Baker, 1962). Consequently, PMS for greater follicular growth (FSH-like action) in combination with HCG for elevated ovulation rate (LH-like action) might result in maximum ovulation. However, conception was completely abolished in ewes treated with PMS and HCG. Possibly estrogen predominated the uterus which resulted in rapid ova transport through the oviduct. If PMS causes an endogenous release of LH in the ewe, the LH may result in elevated estrogens. HCG injection may also elevate estrogen levels in addition to causing ovulation. The uterus may then be under the influence of an extremely elevated estrogen level. Since it has been reported that the ratio of estrogen to progesterone was important for proper uterine environment (Foote et al., 1957), a small dose of progesterone during estrus may counteract the predominance of estrogen.

A critical period of time probably exists between HCG administration and subsequent ovulation in several species. Dziuk et al.(1964) reported that ovulation in the ewe occurred approximately 25 hours post HCG injection.

Therefore, the time of HCG injection should precede expected ovulation by approximately 25 hours. This would allow HCG to augment the endogenous release of LH thereby elevating ovulation rate of the ewe.

Available literature indicates that progestogen treatment blocks the surge release of LH from the pituitary gland (McCann, 1962; Zimbleman, 1964). Low levels of a progestogen do not normally affect FSH release or the tonic release of LH, but large doses of progestogen have shown a marked reduction in both FSH and LH release (First et al., 1963). The degree of LH blockage appears to be quantitative since pituitary LH in the bovine was elevated with an increase in progestogen dosage (Kiracofe, unpublished). Present literature does not indicate whether pituitary content can be correlated with plasma levels. Increase in pituitary content may indicate decreased release and subsequent storage but this hypothesis is in direct contradiction to that reported by Robinson and Nalbandov (1951). Investigation in the future should be concerned with plasma gonadotropin levels with subsequent treatment with progestogens. Also of interest would be the possibility of determining a correlation between plasma gonadotropin levels and pituitary content.

The use of oral progestogens for estrus synchronization has shown mild success but conception rates immediately following some progestogens have been quite low (Hansel, 1964; Zimbleman, 1964). Possible reasons for unsatisfactory conception may be (1) improper ova or sperm transport, (2) asynchronous estrus and ovulation (3) embryonic mortality (Robinson, 1961). Although conception rates are low at the first service following progestogen treatment, subsequent estrus periods are marked by normal or above normal fertility (Zimbleman, 1964). Consequently, data indicates that treatment with a progestogen has a transient detrimental effect on reproduction primarily affecting fertility at the immediate estrus period.

In determining a hypothesis concerned with low fertility following progestogen treatment, ova ovulated have been reported to be normally fertilizable and the time of estrus onset and ovulation tends to fall within normal limits (Robinson, 1961). Consequently, malfunction in ova transport or improper uterine environment may possibly be the end result of progestogen treatment. FSH and LH ratios were reported to remain constant during the cycle but this ratio may be altered with progestogens. Since LH levels may be decreased, FSH levels may predominate the cycle with subsequent follicular stimulation and estrogenicity (Zimbleman, 1964). Thus an abnormal ratio of estrogen to progesterone may result in an improperly developed uterine environment. Zimbleman (1964) observed signs of estrogenicity such as increased uterine tone, vulvar swelling and mucous discharge in heifers receiving an oral progestogen. Another possible explanation could be that LH release from the pituitary gland following progestogen withdrawal may be elevated due to the previous storage. Thus the release of estrogen at the onset of estrus may be elevated above that of the normal cycle with a resulting increase in ova transport. Literature indicates that speed of ova transport through the oviduct was enhanced by estrogen and retarded by progesterone treatment (Harrington, 1965; Harper, 1966). The preceding discussion can only be speculative at the present time and serves as only a possible explanation for low fertility following progestogen treatment. Future experimentation may be centered around gonadotropin, estrogen and progesterone levels prior to and immediately after progestogen cessation for more conclusive evidence.

The use of an oral progestogen in combination with flushing, PMS and HCG may warrant merit in the future but at the present insufficient evidence exists for a conclusive decision. From the present experiment, the number of ewes exhibiting estrus following progestogen treatment was significantly

increased by flushing. Since flushing has not always resulted in elevated ovulation rate (Clark, 1935), the use of exogenous gonadotropins may be an additional benefit. PMS treatment may be sufficient if the time of administration corresponds to the time of normal follicular atresia and ovulation. However, the literature at the present does not indicate whether PMS treatment in species other than the rat results in LH release from the pituitary. PMS and HCG treatment had an adverse affect on conception in the present experiment but this effect may be a result of improper time of administration.

With information on the endogenous hormone levels and the effect of various treatments on these levels, the possibility exists that ovulation rate and fertility may be masterfully controlled in domestic animals as well as the human. The conduction of laborious and expensive experiments appears to be the best solution to a multitude of questions.

SUMMARY

Effects of estrus synchronization, flushing and exogenous gonadotropin treatment on ovulation rate was observed in mature cycling whiteface western ewes. Ewes were fed either a flushing or maintenance ration for 17 days prior to breeding. The progestin, MAP, 60 mg per ewe daily, was added to the pelleted rations. PMS, 750 IU, was administered subcutaneously on day 15 to one-half of the ewes receiving either of the two rations and 500 IU of HCG was injected into one-half of the PMS treated and one-half of the non-treated ewes on day 19. Ewes were bred at the first estrus following MAP treatment. Ewes returning to estrus were laparotomized 48 to 72 hours subsequently while non-return ewes were laparotomized on day 26 of pregnancy to determine ovulation rate following the various treatments.

MAP prevented estrus during the 17 day feeding period in both lots as confirmed by the presence of a "paint-marked" vasectomized ram.

Flushing significantly ($P < .01$) increased weight gain and number of ewes exhibiting estrus following MAP treatment but had no effect on time from post MAP treatment to estrus. Flushing increased ovulation rate and number of ewes lambing above non-flushed ewes but was not significant ($P > .05$). It was postulated that the mechanism of flushing was one of acting upon (1) the releasing factors in the hypothalamus, (2) direct effect on the anterior pituitary gland or (3) increased end organ sensitivity. Available evidence indicates that flushing may stimulate the releasing factors from the hypothalamus which affect the gonadotropic output from the anterior pituitary.

PMS treatment suppressed weight gain and had a detrimental affect on ovulation rate and number of ewes lambing. Although the adverse effect of PMS treatment cannot be fully explained, improper time of administration may have been involved.

HCG treatment increased ovulation rate in treated ewes but decreased the number of ewes lambing. Again, the reason for the adverse affect on conception is unknown. Hormonal imbalance and subsequent asynchronous estrus and ovulation could account for the detrimental effects.

Number of ewes lambing were too few for valid conclusions on lambing rate. Average number of lambs born of ewes lambing was 1.4.

ACKNOWLEDGEMENTS

The author wishes to express sincere gratitude to his major professor and friend, Dr. H. G. Spies, for his devoted personal concern, guidance and assistance during the course of study and preparation of this thesis.

Acknowledgement is extended to Dr. Carl Menzies for his suggestions as well as providing the facilities necessary to conduct this study.

The author also extends appreciation to Douglas J. Bolt and Dr. Guy Kiracofe for their laborious assistance and criticism.

Appreciation of the faithful devotion and understanding of the author's wife, Linda, cannot be over emphasized.

BIBLIOGRAPHY

Bellows, R. A., A. L. Pope, R. K. Meyer, A. B. Chapman and L. E. Casida. 1963. Physiological mechanisms in nutritionally-induced differences in ovarian activity of mature ewes. An. Sci. 22:93.

Bond, J., J. N. Wiltbank and A. C. Cook. 1958. Cessation of estrus and ovarian activity in a group of beef heifers on extremely low levels of energy and protein. J. An. Sci. 17:1211 (Abstract).

Brunner, M. A. et al. 1964. Use of 6-methyl-17-acetoxy-progesterone and PMS to induce and synchronize estrus in ewes. J. An. Sci. 23:32.

Campbell, F. R., J. H. Jones and W. T. Hardy. 1959. Response of range ewes to flushing. Tex. Agr. Exp. Stat. Prog. Rpt. 2111.

Christian, R. E. and J. C. Nofziger. 1952. Puberty and other reproductive phenomena in gilts as affected by plane of nutrition. J. An. Sci. 11:789 (Abstract).

Clark, R. T. 1934. Studies on the physiology of reproduction in the sheep. I. The ovulation rate of the ewes as affected by the plane of nutrition. Anat. Rec. 60:125.

Clark, R. T. 1935. Studies on the physiology of reproduction in the sheep. Anat. Rec. 60:126.

Cole, H. H. and G. H. Hart. 1930. The potency of blood serum of mares in progressive stages of pregnancy in effecting the sexual maturity of the immature rat. Am. J. Physiol. 93:57.

Cole, H. H., G. H. Hart and R. F. Miller. 1945. Studies on the hormonal control of estrous phenomena in the anestrous ewe. Endo. 36:370.

Combs, Weslie, M. P. Botkin and G. E. Nelms. 1961. Synchronization of estrus and lambing in ewes fed 6-methyl-17-acetoxy-progesterone. J. An. Sci. 20:968 (Abstract).

Darroch, J. G., A. W. Nordskag and J. L. Van Horn. 1950. The effect of feeding concentrates to range ewes on lamb and wool productivity. J. An. Sci. 9:431.

Darwash, O., G. B. Marion and H. T. Gier. 1965. Effects of melengesterol acetate on bovine reproductive cycles. J. An. Sci. 24:915. (Abstract).

Day, B. N., L. L. Anderson, L. N. Hazel and R. M. Melampy. 1959. Synchronization of estrus and ovulation in swine. J. An. Sci. 18:909.

Dutt, R. H. 1953. Induction of estrus and ovulation in anoestrual ewes by use of progesterone and PMS. J. An. Sci. 12:515.

Dziuk, P. J. and R. D. Baker. 1961. Control and synchronization of ovulation in swine. J. An. Sci. 20:969 (Abstract).

Dziuk, P. J. and R. D. Baker. 1962. Induction and control of ovulation in swine. J. An. Sci. 21:697.

Dziuk, P. J., F. C. Hinds, M. E. Mansfield and R. D. Baker. 1964. Follicle growth and control of ovulation in the ewe following treatment with 6-methyl-17-acetoxyprogesterone. J. An. Sci. 23:787.

El Sheikh, A. S., C. V. Hulet, A. L. Pope and L. E. Casida. 1955. The effect of level of feeding on the reproductive capacity of the ewe. J. An. Sci. 14:919.

Ershoff, B. H. 1952. Nutrition and the anterior pituitary with special reference to the general adaptation syndrome. Vitamins Hormones (NY) 10:79.

Evans, J. S., R. H. Dutt and E. C. Simpson. 1962. Breeding performance in ewes after synchronized estrus by feeding 6-methyl-17-acetoxyprogesterone. J. An. Sci. 21:804.

First, N. L., F. W. Stratman, E. M. Rigor and L. E. Casida. 1963. Factors affecting ovulation and follicular cyst formation in sows and gilts fed 6-methyl-17-acetoxyprogesterone. J. An. Sci. 22:66.

Foote, W. C., A. L. Pope, A. B. Chapman and L. E. Casida. 1957. Some effects of level of feeding on ovulation rate in yearling ewes. J. An. Sci. 16:1100 (Abstract).

Foote, W. D., L. D. Gooch, A. L. Pope and L. E. Casida. 1957. The maintenance of early pregnancy in the ovariectomized ewe by injection of ovarian hormones. J. An. Sci. 16:986.

Foote, W. C., D. P. Waldorf, H. L. Self and L. E. Casida. 1958. Some effects of progesterone and estradiol on the ovarian structures and on gonadotrophic potency of the pituitary gland in the gilt. J. An. Sci. 17:534.

Foote, W. C., A. L. Pope, A. B. Chapman and L. E. Casida. 1959. Reproduction in the yearling ewe as affected by breed and sequence of feeding levels. I. Effects on ovulation rate and embryo survival. J. An. Sci. 18:453.

Foote, W. C., A. L. Pope, A. B. Chapman and L. E. Casida. 1959. Reproduction in the yearling ewe as affected by breed and sequence of feeding levels. II. Effects on fetal development. J. An. Sci. 18:463.

Gerrits, R. J. and L. A. Johnson. 1965. Influence of level of ICI 33,828 on synchronization of estrus and embryo survival in sows. J. An. Sci. 24:918 (Abstract).

Gossett, J. W. and A. M. Sorensen, Jr. 1959. The effect of two levels of energy and seasons on reproductive phenomena of gilts. J. An. Sci. 18:40.

Gossett, J. W., G. H. Kiracofe, P. P. Graham and B. Baker. 1963. Effects of equine gonadotropin on ewe reproductivity. Virginia Agr. Exp. Stat. Bull. 164.

Haines, C. E., A. C. Warnick and H. D. Wallace. 1959. The effect of two levels of energy intake on reproductive phenomena in Duroc Jersey gilts. J. An. Sci. 18:347.

Haines, C. E. and A. C. Warnick. 1959. The gonadotrophic content of pituitary glands from gilts at two stages of early pregnancy and two levels of energy intake. J. An. Sci. 18:355.

Hamond, J. 1921. Further observations on the factors controlling fertility and foetal atrophy. J. Agr. Sci. 11:337.

Hansel, W., P. V. Malven and D. L. Black. 1961. Estrous cycle regulation in the bovine. J. An. Sci. 20:621.

Hansel, W. 1964. Evaluations of methods for controlling the estrous cycle. Conference on estrous cycle control in domestic animals. Univ. of Nebraska. July 1964.

Harper, M. J. K. 1966. Hormonal control of transport of eggs in cumulus through the ampulla of the rabbit oviduct. Endo. 78:568.

Harrington, F. E. 1965. Transportation of ova and zygotes through the genital tract of immature mice treated with gonadotropins. Endo. 77:635.

Hinds, F. C., P. J. Dziuk and J. M. Lewis. 1964. Control of estrus and lambing performance in cycling ewes fed 6-methyl-17-acetoxyprogesterone. J. An. Sci. 23:782.

Hogue, D. E., Wm. Hansel and R. W. Bratton. 1961. Fertility of ewes bred naturally and artificially after estrus cycles synchronized with an oral progestational agent. J. An. Sci. 20:972 (Abstract).

Hogue, D. E., W. Hansel and R. W. Bratton. 1962. Fertility of ewes bred naturally and artificially after estrous cycle synchronization with an oral progestational agent. J. An. Sci. 21:625.

Howland, B. E., R. L. Kirkpatrick, A. L. Pope and L. E. Casida. 1965. Ovarian activity of ewes on two nutritional levels. J. An. Sci. 24:920.

Hulet, C. V., R. L. Blackwell, S. K. Ercanbrack, D. A. Price and R. D. Humphrey. 1962. Effects of feed and length of flushing period on lamb production in range ewes. J. An. Sci. 21:505.

Joubert, D. M. 1954. The influence of high and low nutritional planes on the oestrus cycle and conception rates of heifers. J. Agr. Sci. 45:164.

Lamond, D. R. 1964. Quantitative studies of the interaction between progesterone and pregnant mare serum on ovarian function in the ewe. J. Reprod. and Fert. 7:171-183 (Apr.).

McCann, S. M. 1962. Effect of progesterone on plasma luteinizing hormone activity. Am. J. of Physiol. 202:601.

Meites, Joseph and Nicholas J. Fiel. 1965. Effect of starvation on hypothalamic content of "somatotropin releasing factor" and pituitary growth hormone content. Endo. 77:455.

Menzies, C. S. and Evans Banbury. 1963. Investigations of milk-fat lamb production practices for western Kansas. Kansas Agr. Exp. Stat. Bull. 473:10.

Moore, N. W. and J. N. Shelton. 1964. Response of the ewe to a horse anterior pituitary extract. J. Reprod. Fert. 7:79-87 (Feb.).

Morrison, F. B. 1959. Feeds and Feeding (22nd ed). Morrison Publishing Co. Clinton, Iowa.

Mulinos, M. G. and L. Pomerantz. 1940. Pseudo-hypophysectomy. J. Nutr. 19:493.

Nellor, J. E. 1960. Control of estrus and ovulation in gilts by orally effective progestational compounds. J. An. Sci. 19:412.

Nelms, G. E. and Weslie Combs. 1961. Estrus and fertility in beef cattle subsequent to the oral administration of 6-methyl-17-acetoxyprogesterone. J. An. Sci. 20:975 (Abstract).

Neville Jr., W. E. and M. W. Neathery. 1964. Effect of pregnant mare serum and triiodothyronine on percent lamb crop. J. An. Sci. 23:301 (Abstract).

Parlow, A. F., L. L. Anderson and R. M. Melampy. 1964. Pituitary follicle-stimulating hormone and luteinizing hormone concentration in relation to reproductive stages of the pig. Endo. 75:365.

Pond, W. G., W. Hansel, J. A. Dunn, R. W. Bratton and R. H. Foote. 1965. Estrous cycle synchronization and fertility of gilts fed progestational and estrogenic compounds. J. An. Sci. 24:536.

Pursel, V. G. and E. F. Graham. 1962. Induced estrus in anestrus ewes by use of progestogens and follicle stimulating hormone. J. An. Sci. 21:132.

Quinn, D. L. and M. X. Zarrow. 1964. Inhibition of pregnant mare's serum-induced ovulation in the immature rat. Endo. 74:309.

Ray, D. E. and J. W. McCarty. 1965. Effect of temporary fasting on reproduction in gilts. J. An. Sci. 24:660.

Robertson, G. L., L. E. Casida, R. H. Grummer and A. B. Chapman. 1951. Some feeding and management factors affecting age at puberty and related phenomena in Chester White and Poland China gilts. J. An. Sci. 10:841.

Robinson, G. E. Jr. and A. V. Nalbandov. 1951. Changes in the hormone content of swine pituitaries during the estrual cycle. J. An. Sci. 10:469.

Robinson, T. J. 1951. The control of fertility of sheep. Part II. The augmentation of fertility by gonadotrophin treatment of the ewe in the normal breeding season. J. Agri. Sci. 41:6.

Robinson, T. J. 1955. Endocrine relationships in the induction of oestrus and ovulation in the anestrous ewe. J. Agri. Sci. 46:37.

Robinson, T. J. 1961. The time of ovulation and efficiency of fertilization following progesterone and pregnant mare serum treatment in the cyclic ewe. J. Agri. Sci. 57:129.

Self, H. L., R. H. Grummer, C. E. Hays and H. G. Spies. 1960. Influence of three different feeding levels during growth and gestation on reproduction weight gains and carcass quality in swine. J. An. Sci. 19:274.

Snedecor, G. W. 1956. Statistical Methods (5th ed.). Iowa State College Press, Ames, Iowa.

Sorensen, A. M. Jr., W. B. Thomas and J. W. Gossett. 1961. A further study on the influence of level of energy intake and season on reproductive performance of gilts. J. An. Sci. 20:347.

Stratman, F. W. and N. L. First. 1965. Estrus inhibition gilts fed a dithio-carbamaylhydrazine (ICI 33,828). J. An. Sci. 24:930.

Wagner, J. F. and L. L. Bush. 1961. Orally active progestin in the hormonal control of reproductive activity in the cycling and anestrous ewe. J. An. Sci. 20:980 (Abstract).

Wagner, J. F. and R. N. Seerley. 1961. Synchronization of estrus in gilts with an orally active progestin. J. An. Sci. 20:980 (Abstract).

Warnick, A. C., E. L. Wiggins, L. E. Casida, R. H. Grummer and A. B. Chapman. 1951. Variation in puberty phenomena in inbred gilts. J. An. Sci. 10:479.

Wiltbank, J. N., A. C. Cook, R. E. Davis and E. J. Warwick. 1957. The effect of various combinations of energy and protein on the occurrence of estrus, length of the estrous period and time of ovulation in beef heifers. J. An. Sci. 16:1100 (Abstract).

Wiltbank, J. N., W. W. Rowden, J. E. Ingalls, K. E. Gregory and R. M. Koch. 1962. Effect of energy level on reproductive phenomena of mature Hereford cows. J. An. Sci. 21:219.

Wiltbank, J. N., W. W. Rowden, J. E. Ingalls and D. R. Zimmerman. 1964. Influence of post-partum energy level on reproductive performance of Hereford cows restricted in energy intake prior to calving. J. An. Sci. 23:1049.

Wyss, H. I. and G. Pincus. 1964. Effect of PMS, Estradiol and progesterone on superovulation in the immature rat. Endo. 75:586.

Zarrow, M. X., J. M. Yochim and J. L. McCarthy. 1964. Experimental Endocrinology. Academic Press. New York. p. 301.

Zimbelman, R. G. 1964. Evaluation of some methods for controlling the bovine estrous cycle. Conference on Estrous Cycle Control in Domestic Animals. Univ. of Nebraska. July 1964. p. 17.

Zimmerman, D. R., H. L. Self and L. E. Casida. 1957. The effect of flushing for various lengths of time on the ovulation rate of Chester White and Chester White-Poland China crossbred gilts. J. An. Sci. 16:1099.(Abstract)

Zimmerman, D. R., H. G. Spies, H. L. Self and L. E. Casida. 1960. Ovulation rate in swine as affected by increased energy intake prior to ovulation. J. An. Sci. 19:295.

EFFECT OF FLUSHING AND EXOGENOUS GONADOTROPIN TREATMENT ON REPRODUCTIVE PERFORMANCE OF ESTRUS SYNCHRONIZED MATURE EWES

by

PETER C. HOPPE

B. S., California State Polytechnic College, 1964

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Animal Husbandry

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1966

ABSTRACT

This experiment was conducted to obtain preliminary evidence on estrus synchronization and elevation of ovulation rate with flushing in conjunction with PMS (Pregnant Mare's Serum) and HCG (Human Chorionic Gonadotropin) treatment in mature ewes.

Forty-eight cycling whiteface western ewes were divided into two lots of 24 ewes each. Lot I received a pelleted maintenance ration (1.39 lb TDN) for 17 days prior to breeding while lot II ewes received a pelleted high concentrate ration (2.5 lb TDN) for an equal period of time. The progestin, Repromix, MAP in soybean oil meal, was fed orally in the daily allotted pelleted ration at a level of 60 mg per ewe. On day 15, 12 ewes, one-half the total number, from each lot were injected subcutaneously with 750 IU PMS. MAP treatment was discontinued on day 17. On day 19, 12 ewes, six PMS treated and six non-treated, in both lots were injected intramuscularly with 500 IU HCG.

Ewes exhibiting estrus following MAP treatment were hand mated to fertile rams. Ewes not exhibiting estrus were laparotomized 48 to 72 hours after detection of subsequent estrus to determine ovulation rate. Twenty-six days post-breeding, non-return ewes were laparotomized to verify pregnancy and count corpora lutea.

Flushed ewes significantly ($P < .01$) increased weight gain over non-flushed ewes. PMS treatment suppressed weight gain in both lots and the difference approached significance ($P < .08$). MAP prevented estrus in all ewes over the 17 day feeding period as evidenced by no "paint-marks" from vasectomized rams. Flushing, irrespective of other treatments, significantly ($P < .05$) increased the number of ewes exhibiting estrus (96% vs. 75%) but had no apparent effect on

the time from post MAP treatment to estrus.

Flushing alone and HCG treatment increased ovulation rate above non-flushed ewes while PMS treatment had detrimental effects on ovulation rate. Differences in ovulation rate with various treatments were not significant ($P > .05$).

Flushing increased the number of ewes lambing but PMS, HCG and the combination of PMS and HCG had detrimental effects on lambing.

Average number of lambs born of ewes lambing was 1.4 but number of ewes lambing were too few for valid conclusions.