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Title REPRODUCTION IN CHINCHILLA: SUPEROVULATION

Abstract approved
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The object of this study was to investigate the possibilities of inducing superovulation in the chinchilla (Chinchilla lanigera) by establishing the optimum dosage of pregnant mares' serum (PMS) to produce a maximum ovulatory response, and to compare chinchilla with other animals in this respect. Non-pregnant female chinchillas were given a single subcutaneous injection of 1.0, 1.2, 1.5, 1.6, 1.8, 2.0, 3.3, 4.0, 8.0 or 16.0 C-N units of PMS. The ovaries of these females were examined to determine the effect of PMS on ovarian weight, on the number of small follicles, large follicles, accessory corpora lutea, and corpora lutea of ovulation. The data were compared with those of Hillemann, Tibbitts, and Gaynor (1959) for untreated chinchillas. Following PMS treatment an increase was observed in ovarian weight, in the number of small follicles, accessory corpora lutea, and corpora lutea of ovulation, but not in the number of large follicles. The higher PMS doses induced also an increase in the number of cystic follicles. In terms of over-all ovarian response, and especially in terms of the ovulatory response, it appears that the optimum PMS dose for chinchillas is 1.6 C-N

units. From an analysis of the percent of animals exhibiting an increase in ovulatory response, and of the extent of ovulation in individual animals, it appears that the best response is achieved when PMS is given in the follicular phase of the estrous cycle. Evidently anestrous females cannot be stimulated to ovulate with PMS. A number of other questions requiring further clarification are discussed.

REPRODUCTION IN CHINCHILLA: SUPEROVULATION

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REPRODUCTION IN CHINCHILLA: SUPEROVULATION

INTRODUCTION

Efforts to induce estrus, ovulation, and superovulation have been carried on for many years and in many animals. Smith and Engle (1927) demonstrated the existence of an anterior pituitary-gonadal relationship with respect to ovulation, and Engle (1927) demonstrated (in mice) that superovulation could be accomplished with gonadotropins. The early attempts at induced ovulation and superovulation involved the use of anterior pituitary transplants from a variety of animals, including mice, rats, rabbits, guinea-pigs, cats, pigs, sheep, horses, and pigeons. Hellbaum (1933) assayed the gonadotropic activity of horse pituitary glands and found these glands to have four times the activity of sheep pituitaries.

Cole and Hart (1930a and 1930b) found that pregnant mares' serum (PMS) collected at certain stages of pregnancy was effective in stimulating the genital systems of immature rats. Cole, Guibert, and Goss (1932) found that 500 rat units (R.U.) of PMS would stimulate non-ovulating female rats to ovulate, and one rat unit (R.U.) would induce ovulation in immature female rats. Their rat unit (R.U.) was defined as follows:

> "One rat unit is the amount of PMS which will produce, in a group of six rats, an average of from three to ten mature follicles or corpora for each immature female rat tested and autopsied five days after the injection, and one-half of which amount will fail to consistently produce a vaginal smear of oestrum in another group of six rats".
> (Cole, Guibert, and Goss, 1932).

The question arose concerning the site of PMS production in the pregnant mare. Hart and Cole (1933) found high concentrations of PMS in the endometrium and in the fetal placenta over the same period during which it was found in the blood stream. Catchpole and Lyons (1934) tested extracts of maternal blood, chorionic fluid, and both maternal and fetal hypophyses for PMS potency; they found that the endometrium was the most potent, and that PMS appeared in the endometrium at the same time as it did in the blood. In further studies, Cole and Goss (1943) and Cole and Hart (1948) determined that PMS was secreted by the endometrial cups, and found high concentrations of PMS in the allantochorionic pedunculated pouches at their points of contact with the endometrial cups.

Cole and Hart (1934) found evidence for the existence of two gonadotropic substances in PMS. One of these substances was present in both pregnant and in non-pregnant mares' serum; it appeared to be a luteinizing substance. The other substance, found only in the serum from pregnant mares, acted as a follicle stimulating substance. Swyer (1964) found PMS to possess predominantly properties similar to those of the follicle stimulating hormone (FSH), along with some properties similar to those of the luteinizing hormone (LH). Numerous other investigators have reported that PMS acts primarily as a follicle stimulating agent. However, McCormack and Meyer (1962) reported evidence to the effect that the ovulation brought about by PMS is due to a certain inherent luteinizing property of the PMS molecule itself, along with the ability of PMS to cause the release of endogenous gonadotropins. It was found by Snook and

Cole (1964) that the luteinizing potential of mares' serum can be increased by injecting FSH as an antigen.

Cartland and Nelson (1937) extracted PMS from crude plasma and obtained a 130-fold increase in purification. Cartland and Nelson (1938) also bioassayed PMS by the ovarian and uterine weight methods. They found the ovarian weight changes to be more quantitative than the uterine weight changes. With this in mind, they defined a dosage quantity which they called a rat unit; this has since been known as the Cartland-Nelson (C-N) unit. They defined their rat unit as follows:

> "One rat unit is equal to the minimum total dose of hormone which, administered to 21 day old rats weighing 35-45 gm in three equal subcutanepus injections at daily intervals, will produce at autopsy, 96 hours after the first injection, a mean ovarian weight of 65 mgm. which is four to five times that of the controls" (Cartland and Nelson, 1938).

Since the discovery of PMS as an ovulation inducing agent, this substance has been tested on many vertebrates. Much of the more practical effort along this line of investigation has been centered on domestic farm animals. Cole and Miller (1933) first produced both estrus and superovulation in ewes with PMS. Robinson (1953) and Hunter, Bishop, and Brown (1958) were able to induce coincident superovulation and estrus in anestrus ewes with 1,000 International Units (I.U.) of PMS if first primed with progesterone (one I.U. of PMS is equivalent to the activity of 0.25 mg. of the International Standard Powder of PMS). However, Foot and Hulet (1964) found that the injection of PMS alone, of progesterone alone, of estradiol alone, or certain combinations of two or three of these substances, resulted in a decrease in the incidence of ovulation of cycling ewes. Superovulation has been

accomplished in goats also (Folley and Greenbaum, 1949) by the use of a threshold dose of 600 I.U. of PMS. However, to produce superovulation and estrus, a dose of at least 1,000 I.U. was required.

Heitman and Cole (1956) produced estrus in lactating sows with 1,120 I.U. and 3,400 I.U. of PMS. But Gibson et al. (1963) were unable to induce superovulation in non-lactating sows with PMS only, even with doses as high as 1,000 I.U. They found that an additional injection of human chorionic gonadotropin (HCG) was required to achieve superovulation. However, Hunter (1964) achieved complete ovulation in sows, whether or not LH was given following PMS treatment.

Attempts to induce superovulation in cattle have been very numerous; it was first accomplished by Casida, McShan, and Wisnicky (1943) by the use of pituitary extracts. Folley and Malpress (1944) successfully induced ovulation in cattle when PMS was given during the follicular phase of the estrous cycle. Hammond and Bhattacharya (1944), Hammond (1949), and Rowson (1951) experienced better ovulatory response to PMS following either the enucleation of the corpora lutea, or by the injection of LH in addition to PMS. Hammond (1949) obtained a better response also when PMS was given near the end of the follicular phase of the estrous cycle. An increase in the incidence of pregnancy was achieved by Luktuke and Bhattacharya (1948) with the injection of PMS. Hafez (1962), Hafez and Sugie (1962), and Hafez, Sugie, and Gordon (1963) carried out a series of studies which involved the injection of PMS followed by HCG. They (1) obtained greater ovulatory response with enucleation

of the corpora lutea, (2) found that they could lengthen physiological estrus but not the intensity of behavioral estrus, (3) discovered that there was no difference in the ovulatory response between left and right ovaries, and (4) noted that there was no advantage in spreading the total dose of PMS over a period of several days.

The effects of PMS have been studied in an additional variety of animals. Asmundson and Wolf (1935) and Breneman (1936) injected PMS into male and female chicks; they observed an increase in testicular and ovarian weights, comb enlargement, and larger duct systems. Engle and Hamburger (1935) injected rhesus monkeys with PMS, and observed growth and proliferation of the granulosa cells of medium and large follicles with a consequent (and immediate) appearance of "estral" changes in the sex skin. Will (1962) obtained satisfactory responses in human patients with menstrual dysfunction and sterility due to anovulatory cycles through treatment with PMS and HCG, or with estrogenic and progestational hormones. Sluiter, Bels, and Van Oordt (1952) injected PMS, HCG, or both, into female bats (Myotis myotis) and observed (1) the maturation of follicles, (2) the luteinization of primary and secondary follicles, (3) the proliferation of the uterine endometrium, and (4) an increase in ovarian size. Hammond (1952) demonstrated that PMS will induce follicular growth and ovulation in anestrous mink at pelting time. Windle (1939), and Sawyer and Everett (1953) found that PMS would promote follicular growth in cat ovaries.

Most of the superovulation studies involving PMS have been

carried out on rodents and lagomorphs, and especially on rats and mice among the rodents. The works of Cole and Hart (1930), Cole, Guibert, and Goss (1932), and Cartland and Nelson (1937 and 1938) have been mentioned above. Evans, Gustus, and Simpson (1933) assayed the concentration of PMS in pregnant mares' serum by noting its effect on ovarian, testicular, and vesicular gland weights. Rowlands (1944), McCormack and Meyer (1962), Zarrow and Quinn (1963), and Wyss and Pincus (1964) were able to induce superovulation in immature rats with PMS alone, or in combination with HCG. Rowlands and Williams (1943), McCormack and Meyer (1962), and Zarrow and Quinn (1963) working with hypophysectomized rats, found that PMS alone would initiate follicular growth only, and that either HCG or LH must be given in addition to the PMS in order to effect ovulation. These studies revealed that the pituitary gland is necessary to produce endogeneous LH for the release of ova following the injection of PMS alone.

Results similar to these obtained with rats have been obtained with mice also. Saunders (1947) was able to induce ovulation in the diestrous mouse by injecting PMS and pregnancy urine (PU). Fowler and Edwards (1957) and Lamond (1960) were able to induce superovulation in both adult and immature mice with injections of PMS and HCG. Edwards and Fowler (1959) had no difficulty with the induction of estrus and ovulation in cyclic adult mice at any time, although metestrous mice ovulated more ova with very low doses of PMS than mice in other stages of estrus. Falconer et al. (1961) found essentially no difference in the number of eggs ovulated between

the right and left ovaries of either superovulated or untreated mice. Fowler and Edwards (1957) found that 75 percent of the mice treated with PMS and HCG would mate in response to injections of these gonadotropins. However, the mean litter size of these treated females was not increased above that found after natural mating. In a later study Edwards and Fowler (1960) found that mice treated with both PMS and HCG resumed natural estrous cycles within three to six days, and that subsequent fertility was unimpaired by this treatment.

One of the first attempts to induce ovulation with PMS in rabbits was achieved by Pincus (1940). He failed to induce superovulation in either prepubertal or pubertal rabbits, apparently due to the formation of cystic follicles in the ovary; he was able to induce superovulation when using pituitary extracts. Parks (1943) used PMS to induce follicular growth in rabbits; ovulation and luteinization was accomplished by a subsequent injection of urinary gonadotropin. Lloyd (1951) demonstrated that PMS would induce extensive proliferation of thecal cells in medium-sized follicles, but she observed no evidence of corpora lutea or follicular luteinization. Hafez (1964) studied seasonal fluctuations in both the ovulation rate and superovulatory response of domestic rabbits; he found no consistent seasonal variations in the superovulatory response, but the response generally tended to be higher in the spring.

A number of studies have been carried out concerning the effects of PMS on hamsters and guinea-pigs. Bodemer, Rumery, and Blandau

(1959) and Bodemer and Warnick (1961) were able to induce superovulation in 31- to 36-day old hamsters with PMS followed by interstitial cell stimulating hormone (ICSH) and noted that this treatment resulted in an increase in the number of polyovular follicles. Greenwald (1962) found that five I.U. of PMS would prevent follicular atresia, and that 30 I.U. would mature both reserve follicles and normally developing follicles in adult hamsters. Hamburger and Pederson-Bjergaard (1946) found that proper dosage levels of PMS would induce an increase in uterine weight, follicular size, and the number of pseudo-corpora lutea in infantile female guinea-pigs. Perry and Rowlands (1963) determined that 25 I.U. of PMS would restore the ovarian weight of hypophysectomized immature guinea-pigs to that of unoperated animals, and that 50-100 I.U. of PMS would cause an increase in ovarian weight above that of unoperated animals.

A rather interesting study was carried out by Wells (1937) on two hermaphroditic ground squirrels (Citellus tridecemlineatus). One animal was used as a control and the other was injected with 25 R.U. of PMS per day for 18 days. In the PMS-treated animal the ovotestes enlarged. The right ovotestis descended into the scrotum, and about 95 percent of its volume was composed of seminiferous tubules. The left ovotestis was about one-half as large as the right; it exhibited a greater amount of ovarian elements and failed to descend into the scrotum. Both ovotestes produced sperm, Graäfian follicles, and corpora lutea. The male and female accessory organs increased in size, and their histological appearance resembled that of normal adult squirrels.

A number of compounds and drugs other than gonadotropins have been used to induce or improve the ovulatory response. Brooks, Beadenkopf, and Bojar (1940), Friedman (1941), and Suzuki and Bialy (1964) have induced ovulation in rabbits with the administration of copper acetate, copper salts, and cupric gluconate. Wilbur and McPhail (1944) were able to facilitate egg release in frogs with sodium fluoride. Greenblatt, Barfield, and Lamprose (1956) found that cortisone was capable of improving cyclic ovarian functions in many women with menstrual abnormalities. Greenblatt et al. (1962) and Naville et al. (1964) were able to induce ovulation in women experiencing certain conditions of ovulatory failure by the administration of clomiphene (a nonsteroidal, non-humoral, synthetic drug). Wilson and Chai (1962) initiated an increase in oval counts and ovarian weight in mice with 0.1 percent propylthiouracil. McCormack and Meyer (1962) were able to increase the percentage of immature rats that would ovulate following PMS treatment through the proper administration of progesterone. However, improper administration of progesterone resulted in the inhibition of ovulation. Hafez, Sugie, and Hunt (1963), working with cattle, found an increase in the number of developing follicles and fertilized ova per cow if estrogen was given between the administration of PMS and HCG. But, if estrogen was given before PMS, then there was a decrease in the number of developing follicles. Attempts were made by Brooks, Beadenkopf, and Bojar (1940) and by Bellows et al. (1962) to improve ovulatory response in rabbits and cattle with the administration of insulin; they found that insulin had no effect. Brooks, Beadenkopf, and

Bojar (1940) found that strychnine sulfate, benzidrine, and ephedrine also have no effect on the initiation of ovulation.

A large number of compounds, hormones, and drugs have also been used to inhibit the action of gonadotropins and ovulation. Purshottam, Mason, and Pincus (1961) and Hopkins and Pincus (1963) inhibited ovulation in mice and rats with the administration of reserpine. Purshottam, Mason, and Pincus (1961) found sparine, trilafon, some steroids, and chlorpromazine also to be inhibitory. Quinn and Zarrow (1964) likewise observed an inhibitory response with chlorpromazine. McCormack and Meyer (1962 and 1963) inhibited the effect of PMS on rats with barbital sodium. Zarrow and Quinn (1963) and Quinn and Zarrow (1964) inhibited the PMS-induction of ovulation in rats with atropine, nembutal, and N-(9-fluorenyl)-N-ethyl-B-chloroethylamine hydrochloride. Zarrow and Quinn (1963) obtained inhibition of ovulation with dibenamine also. Wilson and Chai (1962) observed a reduction in the average number of ova released by rats fed thyroxine. Brown-Grant, Quinn, and Zarrow (1964) found that androgen-treated rats failed to ovulate following injections of PMS alone. Norethynoderl, estradiol, and estrone, when given at proper dosage levels, were found to inhibit ovulation in rats (France and Pincus, 1964). Friedman (1941) found that adequate doses of progesterone would inhibit the induction of ovulation in rabbits treated with copper salts. The proper administration of progesterone was also found to inhibit follicular growth, ovulation, and the synchronization of estrus in cattle (Nellor and Cole, 1956) and in ewes (Wagner et al., 1960). Dzuik et al. (1964) found that

6-methyl-17-acetoxy-progesterone would inhibit ovulation and estrus in ewes. Hopkins and Pincus (1964) were able to reduce PMS-induced superovulation in rats with hypothalamic tissue. Greenwald (1963) blocked superovulation in hamsters by injecting anti-PMS serum from rabbits.

The object of this study was to investigate the possibilities of superovulation in the chinchilla (Chinchilla lanigera) with reference to the optimum dosage of PMS that would give a maximum ovulatory response, and to compare chinchilla with other mammals in this physiological response. Additionally, an attempt was made to determine the PMS dosage that would give maximum follicular growth in terms of number of mature follicles.

MATERIALS AND METHODS

Female chinchillas (selected at random) were to receive different doses of PMS (Gonadogen). These animals were fed standard laboratory rabbit pellets, given water ad libitum, and were exposed to artificial lighting continuously.

A tuberculin syringe was used to administer a single subcutaneous injection of PMS to each female. The dosage levels of PMS administered were 1.0, 1.2, 1.5, 1.6, 1.8, 2.0, 3.3, 4.0, 8.0 and 16.0 Cartland-Nelson units, one Cartland-Nelson unit being equivalent to 20 I.U.

The animals were sacrificed at 72 hours after the administration of PMS. Both ovaries were removed and immersed in 0.9 percent saline. The ovaries were then weighed and subsequently fixed in Bouin's solution. After 24 hours in Bouin's fluid, the ovaries were weighed again. The ovaries were then imbedded in paraffin, serially sectioned at 12 microns, mounted on two by three inch slides, and stained with hematoxylin and eosin.

A further word on the weighing of fresh and fixed ovaries is indicated. Since it is more convenient to determine ovarian weights on fixed material than on fresh, it was necessary to determine what effect fixation would have on the reliability of data. As shown in the tables below, fixation shrinkage was great enough to require fresh weights for determining the true changes in ovarian weights in consequence of PMS treatment.

The ovaries were examined to determine the number and

sizes of medium, and large antral follicles, and to ascertain the number and sizes of both accessory corpora lutea and the corpora lutea of ovulation. The size measurements were made with the aid of an ocular micrometer. The data from this study were then compared with those of Hillemann, Tibbitts, and Gaynor (1959) along with their unpublished raw data, all of which were obtained from untreated chinchillas. Their paper of 1959 included a condensation of this raw data kept on file at Oregon State University.

OBSERVATIONS

The normal (untreated) animals to which reference is made below are those examined by Hillemann, Tibiitts, and Gaynor (1959). These chinchillas, along with the animals used in this study, were non-pregnant. This study was primarily concerned with total performance of PMS-treated animals in comparison with untreated animals.

Table I records the ovarian weight ranges, and averages of both fresh and fixed ovaries, from untreated animals, and from animals injected with various doses of PMS. This table demonstrates that the administration of PMS at all dosage levels produced a subsequent increase in the average ovarian weight, and also in both the minimum and the maximum weights of the ovarian weight ranges. These ovarian weight increases were especially noticeable attendent upon the administration of 1.6, 8.0, and 16.0 C-N units of PMS. Table I illustrates also that fixation of the ovaries with Bouin's fluid results in a decrease in ovarian weight with the exception of the ovaries of five animals treated with 16.0 C-N units of PMS; these weight increments following fixation are unexplained. A comparison of fresh and fixed weights of 152 ovaries demonstrated (subsequent to fixation) an average decrease of 2.94 mg. (7.87 percent).

The number and percentage of animals showing an increase in fresh ovarian weight following various doses of PMS, are included in Table II. It is seen that 50 to 100 percent of the animals, injected with any given dose of PMS, exhibited increases in ovarian weight. Therefore, the increase in fresh ovarian weight averages

Table I. Weight ranges and averages (in mg.) of fresh and fixed ovaries, at various doses of PMS.

Dose (C-N)	Number of Animals	Fresh wt. Ranges		Fixed wt. Ranges		Fresh wt. Averages		Fixed wt. Averages	
		Per Ovary	Per Animal	Per Ovary	Per Animal	Per Ovary	Per Animal	Per Ovary	Per Animal
0.0	46	9-45	18-85	---	---	26.91	47.85	---	---
1.0*	10	13.6-53.7	32.1-102.3	10.7-50.8	25.6-91.2	30.98	61.96	28.50	57.00
1.2*	12	11.0-49.7	18.8-105.5	8.1-46.8	16.2-90.4	31.87	62.74	28.62	57.34
1.5	10	6.1-78.8	14.3-105.3	5.7-46.5	13.2-82.3	28.11	56.22	23.37	46.74
1.6	10	19.6-129.5	53.0-215.0	19.2-101.5	52.6-167.0	41.23	82.46	37.68	75.36
1.8	10	11.8-81.4	33.0-144.2	10.1-77.5	32.4-137.8	28.15	56.30	26.92	53.84
2.0*	12	16.8-82.5	42.3-128.0	13.9-79.6	36.5-122.2	33.43	66.86	30.49	60.98
3.3	10	9.3-54.4	22.1-101.8	8.0-52.9	19.1-99.1	26.67	53.34	24.71	49.42
4.0	10	17.4-46.6	38.3-92.5	15.8-46.6	36.8-91.9	28.96	57.92	27.78	55.56
8.0	10	17.9-139.6	38.5-258.2	16.0-114.6	34.1-197.2	47.16	94.32	41.02	82.04
16.0	10	21.7-114.7	59.5-181.4	24.5-121.5	58.9-170.0	46.51	93.02	52.69	105.38

* Since fresh weights were not available for some ovaries in these categories, their individual weights were corrected for shrinkage by the addition of 2.94 mg. (The average amount of shrinkage based on 152 ovaries)

Table II. Number and percent of animals showing an increase above the untreated average of 47.82 mg., and above the untreated maximum of 85 mg., at various doses of PMS.

Dose (C-N)	Number of Animals	Increases of ovarian wts. 0-20% above the ave. wt. of untreated ovaries		Increases of ovarian wts. exceeding 20% of wt. of untreated ovaries		Increases of ovarian wts. above the max. recorded normal ovarian wt. of 85 mg.	
		Number of Animals	Percent of Animals	Number of Animals	Percent of Animals	Number of Animals	Percent of Animals
0.0	46	11	23.91	11	23.91	3	6.52
1.0	10	3	30.00	5	50.00	2	20.00
1.2	12	1	8.33	7	58.33	3	25.00
1.5	10	1	10.00	5	50.00	2	20.00
1.6	10	2	20.00	8	80.00	3	30.00
1.8	10	1	10.00	2	20.00	2	20.00
2.0	12	5	41.67	5	41.67	2	16.67
3.3	10	1	10.00	4	40.00	1	10.00
4.0	10	2	20.00	5	50.00	1	10.00
8.0	10	1	10.00	8	80.00	5	50.00
16.0	10	0	0.00	10	100.00	6	60.00

were due to a participation of most of the PMS-treated animals. The most effective doses, in terms of the percentage of animals experiencing an increase in ovarian weight, were 1.6, 1.8, and 16.0 C-N units. The percentage of animals exhibiting an increase in ovarian weight following these doses were 100, 80, and 100 percent respectively.

In view of the fact that ovarian weight increases have been observed in a variety of animals following PMS treatment (Willett, 1953), a similar response after PMS injection was expected to occur in chinchillas also. Hillemann, Tibbitts, and Gaynor (1959) recorded an increase in the ovarian weights of pregnant chinchillas. It is presumed that their results were due to endogenous hormonal influences on the ovary. One of the early techniques used for PMS bioassay involved the ovarian weight increases observed in rats (Cartland and Nelson, 1938). Hamburger and Pederson-Bjergaard (1946), using 32 I.U. of PMS (or more), noted an increase in the ovarian weights of infantile guinea-pigs of 100 percent over the ovarian weights observed in untreated animals. Rowlands and Williams (1943), with the administration of 40 I.U. of PMS, were able to restore the weights of atrophic ovaries of hypophysectomized rats to weight equal to those of intact pubertal rats. Perry and Rowlands (1963) observed a 50 percent reduction in ovarian weight following hypophysectomy of immature guinea-pigs. They also reported that the administration of 25 I.U. of PMS restored the ovarian weights of these animals to those of unoperated animals, and that 50 I.U. of PMS brought about an increase in ovarian weights

in the hypophysectomized animals.

Table III summarizes the size ranges and size averages of small follicles, of large follicles, of accessory corpora lutea, and of corpora lutea of ovulation; sizes were based on maximum diameters of the structures involved. The size ranges and size averages of the follicles and corpora lutea, following the administration of PMS, were not markedly different from those observed by Hillemann, Tibbitts, and Gaynor (1959) in their animals. A slight increase in the maximum sizes of corpora lutea of ovulation was observed at most PMS dosage levels. However, these increases were exhibited by only one or a few corpora lutea of ovulation at each PMS dosage level. Large follicles and corpora lutea of ovulation were found to be very similar in size, as were also the small follicles and accessory corpora lutea. Such size similarities were also observed by Hillemann, Tibbitts, and Gaynor (1959) in their untreated animals.

As can be seen in Table IV, the average number, and the maximum number of small follicles (per ovary and per animal) increased noticeably above the untreated average and maximum number of small follicles, subsequent to the administration of PMS at all dosage levels. Tables V and X reveal that 60 to 90 percent of the animals, treated with a given dose of PMS, exhibited an increase of 50 percent or more in the number of small follicles. In addition, Table V shows that 25 to 60 percent of the animals, treated with a given dose of PMS, exhibited an increase of 150 percent or more of these follicles; this increased number is equal to, or greater than, the maximum number of small follicles observed in untreated

Table III. Size ranges and averages (in mm.) of small follicles, of large follicles, of accessory corpora lutea, and of corpora lutea of ovulation, at various doses of PMS.

Dose (C-N)	Number of Animals	Small follicles		Large follicles		Accessory corpora lutea		Corpora lutea of ovulation	
		Range	Average	Range	Average	Range	Average	Range	Average
0.0	46	0.20-0.86	0.47	0.55-1.20	0.91	0.20-0.86	0.45	0.80-1.60	1.20
1.0	10	0.19-0.71	0.43	0.71-1.33	0.91	0.14-0.71	0.44	0.71-1.94	1.06
1.2	12	0.24-0.71	0.43	0.71-1.28	0.90	0.14-0.71	0.39	0.71-1.99	1.15
1.5	10	0.19-0.71	0.42	0.71-1.09	0.86	0.19-0.71	0.38	0.71-1.56	1.05
1.6	10	0.24-0.71	0.44	0.71-1.19	0.90	0.19-0.71	0.37	0.71-1.94	0.99
1.8	10	0.24-0.66	0.40	0.66-1.19	0.80	0.19-0.66	0.41	0.66-1.71	0.96
2.0	12	0.14-0.66	0.43	0.71-1.19	0.80	0.14-0.71	0.38	0.76-1.66	1.13
3.3	10	0.24-0.71	0.44	0.71-1.00	0.83	0.19-0.66	0.41	0.57-1.75	1.09
4.0	10	0.24-0.66	0.43	0.57-1.14	0.86	0.14-0.66	0.40	0.71-1.71	1.08
8.0	10	0.14-0.71	0.43	0.71-1.19	0.88	0.14-0.71	0.49	0.81-1.90	1.26
16.0	10	0.19-0.71	0.41	0.71-1.61	0.94	0.19-0.76	0.45	0.76-1.75	1.13

Table IV. Ranges and averages in the number of small and of large follicles, at various doses of PMS.

Dose (C-N)	Number of Animals	Small Follicles				Large Follicles			
		Range		Average		Range		Average	
		Per Ovary	Per Animal	Per Ovary	Per Animal	Per Ovary	Per Animal	Per Ovary	Per Animal
0.0	46	0-19	0-25	4.74	10.06	0- 6	0- 7	1.42	2.84
1.0	10	3-23	6-41	13.65	27.30	0- 3	0- 5	1.00	2.00
1.2	10	1-28	4-39	9.75	18.50	0- 4	0- 7	1.58	3.17
1.5	10	5-33	11-57	15.35	31.00	0- 5	0- 9	1.45	2.90
1.6	10	4-24	8-39	11.95	23.93	0- 5	0- 7	1.55	3.10
1.8	10	0-29	3-51	13.00	25.00	0- 3	2- 4	1.45	2.90
2.0	12	2-30	9-49	11.86	23.74	0- 6	0- 8	1.88	3.75
3.3	10	3-32	7-49	11.20	22.40	0- 5	0-10	2.15	4.30
4.0	10	4-23	12-35	11.70	23.40	0-10	0-17	1.45	2.90
8.0	10	3-31	13-57	14.95	29.90	0-11	2-13	3.95	7.90
16.0	10	4-38	13-62	14.75	29.50	0- 5	2- 9	2.50	5.00

Table V. Number and percent of animals, given various doses of PMS, and subsequently showing an increase in the number of small follicles above the untreated average number of 10.06 follicles.

Dose (C-N)	Number of Animals	15-19 follicles (increase of 50-99%)		20-24 follicles (increase of 100-149%)		25 follicles or more (increase of 150% or more)	
		Number of Animals	Percent of Animals	Number of Animals	Percent of Animals	Number of Animals	Percent of Animals
0.0	46	3	6.52	4	8.70	2	4.35
1.0	10	0	0.00	3	30.00	6	60.00
1.2	12	1	8.33	2	16.67	4	33.33
1.5	10	2	20.00	0	0.00	6	60.00
1.6	10	0	0.00	1	10.00	6	60.00
1.8	10	2	20.00	1	10.00	5	50.00
2.0	12	3	25.00	3	25.00	3	25.00
3.3	10	0	0.00	3	30.00	3	30.00
4.0	10	2	20.00	1	10.00	5	50.00
8.0	10	2	20.00	1	10.00	6	60.00
16.0	10	3	30.00	0	0.00	6	60.00

animals.

The observed increase in the number of small follicles is attributed to the follicle-stimulating activity of PMS, since according to many investigators including Parkes (1943), Hafez, Sugie, and Gordon (1963), and Swyer (1964), follicle-stimulation is the primary function of PMS. Greenwald (1962), working with hamsters, observed an increase in the number of reserve follicles. He found this to be due to an increase in the production of small follicles coupled with a reduction in follicular atresia. Therefore, the follicle-stimulating activity of PMS may initiate an increase in the number of small follicles (1) by stimulating primary, secondary, and tertiary follicles (all non-antral) to form small Graäfian follicles, and (2) by reducing the amount of atresia in small follicles.

The ranges and averages of the number of large follicles did not increase noticeably following the administration of various doses of PMS, with one exception. As noted in Table IV, the administration of 8.0 C-N units of PMS did result in an increase in the average number of large follicles; this increase was greater than 175 percent. The information in Table VI illustrates that the increases in the number of large follicles was restricted to relatively few animals at each dose of PMS. Tables VI and X show that 0 to 41.67 percent of the animals receiving various doses of PMS exhibited an increase in the number of large follicles, with most doses of PMS inducing an increase in the number of large follicles in 10 to 20 percent of the animals treated. The large increase in

Table VI. Number and percent of animals, given various doses of PMS, and subsequently showing an increase in the number of large follicles above the untreated average number of 2.84 follicles.

Dose (C-N)	Number of Animals	5 to 7 follicles (increase of 67-133%)		7 to 9 follicles (increase of 133-200%)		9 or more follicles (increase of 200% or more)	
		Number of Animals	Percent of Animals	Number of Animals	Percent of Animals	Number of Animals	Percent of Animals
0.0	46	5	10.87	2	4.35	0	0.00
1.0	10	1	10.00	0	0.00	0	0.00
1.2	12	1	8.33	1	8.33	0	0.00
1.5	10	0	0.00	0	0.00	1	10.00
1.6	10	1	10.00	1	10.00	0	0.00
1.8	10	0	0.00	0	0.00	0	0.00
2.0	12	3	25.00	2	16.67	0	0.00
3.3	10	0	0.00	0	0.00	2	20.00
4.0	10	1	10.00	0	0.00	1	10.00
8.0	10	1	10.00	0	0.00	1	10.00
16.0	10	2	20.00	1	10.00	1	10.00

the average number of large follicles following the injection of 8.0 C-N units of PMS was primarily due to one animal with 13 large follicles; only 20 percent of the animals treated with this dose showed an increase in the number of large follicles.

It may appear contradictory that an increase in the number of large follicles did not accompany the increase in the number of small follicles, especially if the primary function of PMS is that of follicle-stimulation. However, if the large follicles which may have been produced due to the influence of PMS, are induced to ovulate and undergo luteinization there will not be an increase in the number of large follicles, but there may even be a decrease in the number. Subsequent to this process an increase in the number of corpora lutea of ovulation would occur. It is seen in Table X that very few chinchillas showed an increase in the number of both large follicles and corpora lutea of ovulation. Since large follicle formation with ovulation must precede the formation of corpora lutea, it is probable that PMS treatment (with some doses and in certain animals) did result in an increase in the number of large follicles, but that these follicles ovulated and gave an increase in the number of corpora lutea of ovulation. Ovulation may have been induced by the direct action of PMS or by PMS inducing the release of endogenous LH.

A marked increase in the average number of accessory corpora lutea, above the average number found in untreated animals, was observed subsequent to the administration of nearly all doses of PMS. The only doses of PMS not showing a substantial increase in

number were 2.0 and 8.0 C-N units. It is shown in Table VII that the administration of 2.0 C-N units of PMS gave only a 27.86 percent increase in the number of accessory corpora lutea and that the administration of 8.0 C-N units of PMS resulted in a decrease in the number of these bodies. All other doses of PMS initiated an increase of 50 percent or more, in the average number of accessory corpora lutea. PMS doses of 1.2, 1.5, 1.6, and 1.8 C-N units gave the greatest increases in their number; these four doses initiated an increase of 240 percent or more. A comparison of the information in Table VII with that in Tables VIII and X reveals that the doses of PMS (1.2, 1.5, 1.6, and 1.8 C-N units) which initiated the greatest increases in the average number of accessory corpora lutea, also initiated this increase in the greatest percentage of animals; at these doses, 40 to 67 percent of the animals showed an increase of 100 percent or more, and 60 to 90 percent of the animals showed an increase of 50 percent or more in the number of accessory corpora lutea.

Hillemann, Tibbitts, and Gaynor (1959) surmised that one may gather the impression that the accessory corpora lutea arise by luteinization of small antral and non-antral follicles; their opinion was based largely on the similarity between the sizes of these two structures. Their opinion gains credibility from the fact that both small follicles and accessory corpora lutea increased in number following the PMS treatment in this study. Wolfe and Neigers (1948) noted that certain follicles of human ovaries (subsequent to PMS treatment) exhibited enlarged granulosa cells which were "clear" in

Table VII. Ranges and averages in the number of accessory corpora lutea, and of corpora lutea of ovulation, at various doses of PMS.

Dose (C-N)	Number of Animals	Accessory corpora lutea				Corpora lutea of ovulation			
		Range		Average		Range		Average	
		Per Ovary	Per Animal	Per Ovary	Per Animal	Per Ovary	Per Animal	Per Ovary	Per Animal
0.0	46	0-12	0-24	3.70	6.39	0- 7	0- 9	1.82	3.65
1.0	10	0-18	3-33	4.90	9.80	0- 9	0-15	2.65	5.30
1.2	12	0-27	3-36	9.00	18.00	0- 6	0-10	3.33	6.67
1.5	10	2-19	6-38	8.80	17.60	0-14	0-27	3.50	7.00
1.6	10	2-30	9-51	7.85	15.70	0-16	0-28	4.40	8.80
1.8	10	0-22	5-38	8.45	16.90	0-10	1-18	2.35	4.70
2.0	12	0-29	0-43	4.08	8.17	0- 7	0- 8	2.42	4.83
3.3	10	0-16	0-20	4.75	9.50	0- 5	1- 8	2.00	4.00
4.0	10	1-12	6-23	5.70	11.40	0-11	0-14	2.70	5.40
8.0	10	0- 8	0-11	2.30	4.60	0- 6	0-12	2.20	4.40
16.0	10	2-21	6-24	7.20	14.40	0-12	0-16	3.45	6.90

Table VIII. Number and percent of animals, given various doses of PMS, and subsequently showing an increase in the number of accessory corpora lutea above the untreated average of 6.39 accessory corpora lutea.

Dose (C-N)	Number of Animals	12 to 18 accessory corpora lutea (increase of 100-200%)		18 to 24 accessory corpora lutea (increase of 200-300%)		24 or more accessory corpora lutea (increase of 300% or more)	
		Number of Animals	Percent of Animals	Number of Animals	Percent of Animals	Number of Animals	Percent of Animals
0.0	46	3	6.52	0	0.00	1	2.17
1.0	10	2	20.00	0	0.00	1	10.00
1.2	12	3	25.00	2	16.67	3	25.00
1.5	10	0	0.00	4	40.00	2	20.00
1.6	10	2	20.00	1	10.00	1	10.00
1.8	10	0	0.00	2	20.00	3	30.00
2.0	12	0	0.00	0	0.00	1	8.33
3.3	10	3	30.00	1	10.00	0	0.00
4.0	10	4	40.00	1	10.00	0	0.00
8.0	10	0	0.00	0	0.00	0	0.00
16.0	10	5	50.00	2	20.00	1	10.00

appearance and simulated lutein-type cells; these findings suggested that PMS may induce luteinization of non-ovulating small follicles. Another source of accessory corpora lutea may be the luteinization of small atretic follicles. The luteinization of such follicles (following PMS treatment) was noted by Leathem (1939) in rats, and by Perry and Rowlands (1963) in guinea-pigs. Luteinization of atretic follicles as a source of accessory corpora lutea may not seem likely when one recalls that PMS operates primarily as a follicle-stimulator and as such acts to reduce follicular atresia. However, it seems erroneous to assume that PMS treatment would prevent all small follicles from undergoing atresia. It does, however, seem probable that small follicles which had begun to undergo atresia, prior to or during the administration of PMS, and which were no longer under the influence of endogenous FSH, would be susceptible to the luteinizing activity of the PMS molecule itself, or to endogenous LH.

Several doses of PMS resulted in an increase in the average number of corpora lutea of ovulation above that observed in untreated animals. Table VII illustrates that PMS doses of 1.2, 1.5, 1.6, and 16.0 C-N units initiated an increase of 50 percent or more in the average number of corpora lutea of ovulation. The greatest increase in the average number of corpora lutea of ovulation occurred following the administration of 1.6 C-N units of PMS; at this dose the average number of corpora lutea of ovulation increased 140 percent above the untreated average number. An examination of Tables IX and X reveals that 70 percent of the animals treated with 1.6 C-N units of PMS exhibited an increase of 50 percent or more in the

Table IX. Number and percent of animals, given various doses of PMS, and subsequently showing an increase in the number of corpora lutea of ovulation above the untreated average of 3.65 corpora lutea of ovulation.

Dose (C-N)	Number of Animals	6-8 corpora lutea (increase of 50-100%)		8-12 corpora lutea (increase of 100-200%)		12 or more corpora lutea (increase of 200% or more)	
		Number of Animals	Percent of Animals	Number of Animals	Percent of Animals	Number of Animals	Percent of Animals
0.0	46	4	40.00	2	20.00	0	0.00
1.0	10	2	20.00	2	20.00	1	10.00
1.2	12	1	8.33	7	58.33	0	0.00
1.5	10	0	0.00	3	30.00	1	10.00
1.6	10	3	30.00	3	30.00	1	10.00
1.8	10	1	10.00	0	0.00	1	10.00
2.0	12	4	33.33	2	16.67	0	0.00
3.3	10	2	20.00	1	10.00	0	0.00
4.0	10	2	20.00	2	20.00	1	10.00
8.0	10	0	0.00	1	10.00	1	10.00
16.0	10	4	40.00	2	20.00	2	20.00

number of corpora lutea of ovulation. Table IX shows also that 60 percent of the animals treated with 1.6 C-N units of PMS exhibited an increase of 6 to 12 corpora lutea of ovulation; this is an important point to be discussed below. It should be emphasized that 80 percent of the animals treated with 16.0 C-N units of PMS showed an increase in the number of corpora lutea of ovulation. However, with this dose of PMS, and with a dose of 8.0 C-N units, there was an increase in the number of cystic follicles with retained oocytes. A PMS dose of 1.2 C-N units resulted in 66.67 percent of the animals showing an increase in the number of corpora lutea of ovulation; however, the average increase in the number of these structures at this dose was not as high as that at 1.6 C-N units of PMS. An average of seven corpora lutea per animal was observed following the administration of 1.5 C-N units. However, this increase above the average is accountable on the basis of only four animals, one of which had a high of 27 corpora lutea.

As indicated above, Table X has been used to support the information in Tables V, VI, VIII, and IX. In addition, Table X can be used to demonstrate the existence or absence of relationships among the animals exhibiting an increase in the number of follicles and corpora lutea. Table X demonstrates the existence of a relationship between the number of animals showing an increase in the number of both small follicles and accessory corpora lutea at most doses of PMS; this relationship is especially marked at PMS doses of 1.6 and 16.0 C-N units. Also, a relation exists at PMS doses of 1.6 and 16.0 C-N units, between those animals showing an increase

Table X. Summary of distribution in the number of follicles and of corpora lutea along with the number of animals showing this distribution at various doses of PMS. (This table includes only those animals which show an increase of 50% or more above the average figures for untreated animals.)

Dose		0	1.0	1.2	1.5	1.6	1.8	2.0	3.3	4.0	8.0	16.0
Number of Animals		46	10	12	10	10	10	12	10	10	10	10
No increase in small follicles	No.	35	1	5	2	3	2	3	4	2	1	1
	%	76.09	10.00	41.67	20.00	30.00	20.00	25.00	40.00	20.00	10.00	10.00
An increase in small follicles	No.	11	9	7	8	7	8	9	6	8	9	9
	%	23.91	90.00	58.33	80.00	70.00	80.00	75.00	60.00	80.00	90.00	90.00
No increase in accessory corpora lutea	No.	36	6	3	4	1	4	9	6	4	9	1
	%	78.26	60.00	25.00	40.00	10.00	40.00	75.00	60.00	40.00	90.00	10.00
An increase in accessory corpora lutea	No.	10	4	9	6	8	6	3	4	6	1	9
	%	21.74	40.00	75.00	60.00	80.00	60.00	25.00	40.00	60.00	10.00	90.00
An increase in both small follicles and accessory corpora lutea	No.	6	4	5	4	6	5	3	3	4	1	8
	%	13.04	40.00	41.67	40.00	60.00	50.00	25.00	30.00	40.00	10.00	80.00
No increase in large follicles	No.	40	9	10	9	8	10	7	8	8	8	5
	%	86.96	90.00	83.33	90.00	80.00	100.00	58.33	80.00	80.00	80.00	50.00
An increase in large follicles	No.	6	1	2	1	2	0	5	2	2	2	5
	%	13.04	10.00	16.67	10.00	20.00	0.00	41.67	20.00	20.00	20.00	50.00
No increase in corpora lutea of ovulation	No.	41	5	4	6	3	8	6	9	5	8	2
	%	89.13	50.00	33.33	60.00	30.00	80.00	50.00	90.00	50.00	80.00	20.00
An increase in corpora lutea of ovulation	No.	5	5	8	4	7	2	6	1	5	2	8
	%	10.87	50.00	66.67	40.00	70.00	20.00	50.00	10.00	50.00	20.00	80.00
An increase in both large follicles and corpora lutea of ovulation	No.	0	1	2	1	2	0	2	0	1	1	4
	%	0.00	10.00	16.67	10.00	20.00	0.00	16.67	0.00	10.00	10.00	40.00
An increase in both small and large follicles	No.	1	1	1	1	2	0	5	2	2	2	5
	%	2.17	10.00	8.33	10.00	20.00	0.00	41.67	20.00	20.00	20.00	50.00
An increase in both accessory corpora lutea and corpora lutea of ovulation	No.	1	4	5	1	5	2	2	1	3	0	7
	%	2.17	40.00	41.67	10.00	50.00	20.00	16.67	10.00	30.00	0.00	70.00
An increase in all follicles and corpora lutea	No.	0	1	1	0	2	0	2	0	1	0	4
	%	0.00	10.00	8.33	0.00	20.00	0.00	16.67	0.00	10.00	0.00	40.00

in the number of accessory corpora lutea, and in the number of corpora lutea of ovulation. In addition to illustrating the existence of the above relationships, Table X shows that there are few relationships between the number of animals showing an increase in the number of small and large follicles, and between the number of animals showing an increase in the number of large follicles and corpora lutea of ovulation. Finally, the information in Table X indicates that extremely few animals treated with various doses of PMS, showed a simultaneous increase in the number of all of the ovarian structures mentioned above.

The relationship between the number of small follicles and of accessory corpora lutea, and the increase in the number of corpora lutea of ovulation (following PMS treatment), is related to the general increase in ovarian weight. Hisaw (1947) divided the process of follicular development into four stages: (1) a period of oögenesis, organization of the granulosa and theca interna, and growth up to antral formation; (2) a period of increased follicular competence to respond to the action of pituitary gonadotropins, which competence is marked by rapid follicular growth, by increased mitotic activity in the granulosa and theca interna, and by multiplication of follicular blood vessels; (3) a period of differentiation during which there is a rapid decrease in mitotic activity, a continued increase in vascularization, an increased hypertrophy of granulosa and thecal cells, and an increase in follicle size (due mostly to the accumulation of antral fluid); and (4) a period of continued preovulatory swelling due to rapid secretion of follicular fluid,

and marked hypermia of the follicle. This process of follicle development has been observed (and in many cases accelerated) following PMS treatment in monkeys (Engle and Hamburger, 1935), in man (Wolfe and Neigers, 1948), in rats (Leathem, 1939), in hamsters (Greenwald, 1962), in guinea-pigs (Hamburger and Pederson-Bjergaard, 1946), and in rabbits (Lloyd, 1951). With the formation of an increased number of small follicles (following PMS treatment), there is a subsequent increase in total mitotic activity and hypertrophy in both the granulosa and thecal cells, in vascularization, and in follicular fluid. All of these processes would contribute to an increase in ovarian size and weight. During luteinization there occurs a rapid swelling of the granulosa cells as they fill the vacated antral cavity, an involution of strands of thecal tissue, and a further increase in vascularization. These processes, along with an increase in secretory activity by the corpora lutea, also contribute to an increase in ovarian weight; and with an increase in the number of these structures (following PMS treatment) the contributions of these same items to ovarian weight increase would be magnified. Hamburger and Pederson-Bjergaard (1946) reported a steady increase in stroma cell hypertrophy following high doses of PMS; this hypertrophy may also increase ovarian weight. It should be noted, however, that several investigators have published data indicating that ovarian size by itself is not a satisfactory measure of ovarian response to gonadotropins (Willett, 1953).

The data of Hillemann, Tibbitts, and Gaynor (1959) revealed that the ratios of the number of small to large follicles, of

accessory corpora lutea to corpora lutea of ovulation, and of all follicles to all corpora lutea, in untreated, non-pregnant animals, were 3.5:1, 2.1:1, and 1.2:1 respectively. Table XI is a summary of these same ratios following the administration of different doses of PMS. The information in Table XI indicates that (1) the ratios of the number of small to large follicles increased (following PMS treatment); (2) that the ratios of the number of accessory corpora lutea to corpora lutea of ovulation remained essentially the same as in untreated animals; and (3) that the ratios of the number of all follicles to all corpora lutea increased slightly following the administration of PMS. These ratios are not surprising in view of the fact (1) that there was a general increase in the number of small follicles following the administration of PMS; (2) that there was essentially no increase in the number of large follicles; and (3) that although the number of accessory corpora lutea tended to increase following PMS, there was also a tendency for the number of corpora lutea of ovulation to increase.

From an analysis of the foregoing observations, and of the information contained in the foregoing tables, it appears that the doses of PMS most effective (1) in producing an increase in ovarian weight, (2) in producing an increase in the number of the various ovarian structures mentioned above, and (3) in producing the largest percentage of animals exhibiting these increases, were 1.6 and 16.0 C-N units. The animals receiving these doses of PMS exhibited the best over-all ovarian performance, especially in terms of ovulatory response.

Table XI. Summary of the ratios of small follicles to large follicles, of accessory corpora lutea to corpora lutea of ovulation and of all follicles to all corpora lutea, at various doses of PMS.

Dose (C-N)	Ratio of Small:Large follicles	Ratio of Accessory corpora lutea: Corpora lutea of ovulation	Ratio of All follicles:All corpora lutea
0.0	3.5:1	2.1:1	1.2:1
1.0	13.7:1	1.8:1	1.9:1
1.2	5.8:1	2.7:1	0.9:1
1.5	10.7:1	2.5:1	1.4:1
1.6	7.7:1	1.8:1	1.1:1
1.8	8.6:1	3.6:1	1.3:1
2.0	6.3:1	1.7:1	2.1:1
3.3	5.2:1	2.4:1	1.9:1
4.0	8.1:1	2.1:1	1.6:1
8.0	3.8:1	1.1:1	4.2:1
16.0	5.9:1	2.1:1	1.6:1
Range	3.8:1-13.7:1	1.8:1-3.6:1	0.9:1-4.2:1
Average	7.6:1	2.2:1	1.8:1

DISCUSSION

In order to establish an initial effective ovulatory PMS dose in relation to body weight for chinchilla, reference was made to existing publications relating to both ovulation and superovulation in other animals, and to the recommendations of the manufacturers of PMS (Gonadogen, The Upjohn Co., Kalamazoo, Michigan). On the basis of the minimal ovulatory dose for chinchilla, several increments in the amount of PMS were administered in an effort to determine the dose necessary to achieve a superovulation.

Rowlands and Williams (1943) found the optimum PMS dose for rats to be 40 I.U. However, Rowlands (1944) and Wyss and Pincus (1964) determined that 30 I.U. was the optimum dose for immature rats. Rowlands (1944) noted also that doses greater than this resulted in a regression in the number of ova released, in a regression in the percent of ovulating rats, and in an increase in the number of cystic follicles. McCormack and Meyer (1963) induced 75 percent of immature rats to ovulate with 1.375 C-N units of PMS. An optimum ovulatory response was obtained in hamsters using 30 to 40 I.U. of PMS (Bodemer, Rumery, and Blandau, 1959; Greenwald, 1962). Hamburger and Pederson-Bjergaard (1946) obtained maximum follicular response in infantile guinea-pigs with four to eight I.U. of PMS, and obtained a 100 percent increase in ovarian weight following 32 I.U. Perry and Rowlands (1953), working with hypophysectomized immature guinea-pigs, were able to restore ovarian weights to those of unoperated animals with 25 I.U. In rabbits an optimum ovulatory response was obtained

following the injection of 10 I.U. (Parkes, 1943). Hammond (1952) found the optimum dose for mink to be 100 I.U. The Upjohn Company (Kalamazoo, Michigan), which provided the PMS (Gonadogen) used in this study recommends a dose of two C-N units for mink. Since a consistent relationship exists between PMS dosage optimums and body weights, a comparison was made between the average body weights of the above animals and that of chinchilla. Because the average body weight of chinchillas (500 gm) is between that of rats (250 gm) and mink (1100 gm) it was estimated that the optimum dose for chinchillas should fall between 1.0 and 2.0 C-N units. Higher doses of PMS (3.3, 4.0, 8.0, and 16.0 C-N units) were also administered as a further check and also to determine any effects from these higher doses.

Rowlands and Williams (1943), working with hypophysectomized rats, concluded that PMS must be long acting because they obtained the same effect with a single injection of a given dose, or by dividing that dose into five equal daily injections. Prolonged PMS treatment in rabbits results in a decrease in ovarian stimulation even with a geometric increase in dose; also, this prolonged treatment induces the formation of anti-PMS serum (Parkes, 1943). Hafez, Sugie, and Gordon (1963) noted that an optimum PMS response was effected in cattle following a single injection, and that there was no advantage in spreading the total dose over a period of two to three days. The chinchillas used in this study were given single subcutaneous injections.

Many animals can be induced to ovulate, and to come into estrus regardless of the estrous stage of the animal at the time of PMS

administration. Edwards and Fowler (1959 and 1960) had no difficulty in inducing estrus and ovulation with PMS in cyclic adult mice in any estrous stage, although animals injected during metestrus ovulated more eggs than did those animals treated in other stages. Ovulation may also be induced in rats without regard to the estrous stage in which PMS is given. PMS will induce ovulation and estrus also in noncycling and in cycling hamsters at any time during the estrous cycle (Greenwald, 1962). Cole and Miller (1933) found that ovulation was produced in anestrous ewes when treated with a single injection of PMS, but that a second injection was needed 16 days after the first, to produce both ovulation and estrus. Dutt (1953) also found that a single injection of PMS would induce ovulation, but not estrus, in anestrous ewes. However, Robinson (1956) observed that the injection of PMS would improve the precision of the time of the onset of estrus and ovulation in anestrous ewes. Folley, Greenbaum, and Roy (1949) demonstrated that estrus and ovulation occurred regularly in anestrous goats treated with PMS doses greater than 1,000 I.U. Estrus was produced in lactating sows by Heitman and Cole (1956) with PMS doses of 1,000 to 3,400 I.U. It has also been demonstrated that cows can be superovulated at any stage of the estrous cycle (Willett, 1953). It was anticipated that chinchillas, in any stage of estrus or in anestrus, could also be induced to ovulate, and to come into estrus subsequent to PMS treatment.

In contrast to most of the above observations, some investigators have found that they could not induce ovulation, or both ovulation and estrus at will, with a single injection of PMS, or

with PMS alone. Saunders (1947) observed that only 50 percent of PMS-treated diestrous mice would ovulate. Lamond (1960) demonstrated that it was impossible to obtain ovulation in adult diestrous mice using only a single injection of PMS or HCG, but that ovulation could be induced with PMS when followed in 24 or 48 hours by an injection of HCG. Contrary to Willett's (1953) statement that cows can be induced to ovulate at any stage of the estrus cycle, Folley and Malpress (1944) stated that ovulation followed PMS treatment only when PMS was given during the follicular phase; and Hammond (1949) noted that a better ovulatory response was achieved when normal heat (estrus) was scheduled to follow PMS treatment in three to five days.

The precise length of the estrus cycle (or any of its stages) is not known for the chinchilla. Its duration has been estimated, from information provided by chinchilla ranchers, to be 32 to 40 days (Hillemann, 1960). An attempt during one year was made by Hillemann and Tibbitts (Oregon State University) to plot the estrous cycle by the vaginal smear technique; their attempt was unsuccessful because chinchillas apparently are extremely sensitive to vaginal stimulation, and after the taking of the first vaginal lavage the animals presented a confusing cellular picture suggesting that of pseudopregnancy (personal communication). Since the estrous cycle of the chinchilla is poorly understood, it was not possible to give PMS at preferred stages of the estrous cycle.

A count of the corpora lutea of ovulation was used as the criterion for determining ovulation since an ovulated follicle is represented by a corpus luteum. The number of corpora lutea of

ovulation per animal, along with the percentage of animals exhibiting an increase in the number of these bodies, were thought to indicate the estrous stage in which PMS had been injected. As mentioned above, the most PMS-sensitive stages are the follicular phase (including proestrus and estrus) and metestrus; and the least sensitive stages are diestrus and anestrus. In this study, one to three animals at all PMS doses had a corpora lutea count of zero or one; it is thought that these animals were in anestrus. Several females at all doses exhibited two to five corpora lutea, and it is supposed that these animals were in diestrus. The animals exhibiting a substantial increase in the number of corpora lutea were apparently in the follicular phase, or in metestrus. A few females presented extreme increases in the number of corpora lutea (15 to 28); it is presumed that these females had been treated at the optimum time in the follicular phase for ovulatory response, or that these animals had littered shortly before being treated and with PMS acting to maintain existing corpora lutea. It is concluded that the estrous stage in which chinchillas are given PMS is very important, and that they can not be induced either to come into estrus, or to ovulate at all stages of the estrous cycle or during anestrus. Apparently their PMS response is restricted to selected estrous stages.

The chinchillas used in this study comprised a heterogeneous population of culls from the colonies of chinchilla ranchers. These animalswere culled for various reasons such as: (1) poor reproductive performance, (2) fur chewing, (3) improper fur quality, (4) over age, and (5) poor general condition. One might expect such

a population to present PMS-sensitivity variables. Chapman (1946), working with prepubertal rats, demonstrated that heredity was partially responsible for individual variability in the superovulatory response to gonadotropins; he also estimated that heredity was responsible for as much as 40 percent of the variation in the weight of stimulated ovaries. Hafez (1964) accounted for individual variations in the ovulation rates in rabbits (following PMS treatment) on the basis of heredity, parity, size of the previous litter, period of previous parturition, and physical condition of the animal. Also, Hafez (1964) noted that the ovulatory response to PMS tended to be higher in the spring than during the remainder of the year.

A PMS dose of 1.6 C-N units appears to be the optimum amount to induce superovulation in chinchilla. The average ovarian weight (both ovaries) of the animals treated with this dose was 82.46 mg. (Table I), this weight is 72.32 percent above that for untreated animals. All animals treated at this level showed some increase in ovarian weight (Table II). The increase of 72.32 percent in average ovarian weight is exceeded only by the increases observed at doses of 8.0 and 16.0 C-N units (Table I). Although an increase in ovarian weight by itself is not a measure of ovulatory response, it may be considered as an indication of general ovarian responsiveness to PMS. The animals treated with 1.6 C-N units exhibited an average of 23.93 small follicles (Table IV); this represents an increase of 137.88 percent. Seventy percent of these animals exhibited an increase of 50 percent or more in the number of small follicles (Table V), and of the remaining 30 percent, two animals had 10 small follicles, and one animal had five.

No increase in the number of large follicles was observed following this PMS dose. This fact indicates that nearly all of the large follicles produced, were induced to ovulate. The average number of accessory corpora lutea per animal, treated with 1.6 C-N units, was 15.70 (Table VII); this represents an increase of 145.70 percent. Eighty percent of these animals showed an increase of 50 percent or more in the number of these bodies (Table X), and the remaining 20 percent each had nine accessory corpora lutea (40.84 percent increase). This increase indicates that a dose of 1.6 C-N units induces luteinization either by the action of the PMS molecule itself or by inducing the release of endogenous LH.

As indicated above, the number of corpora lutea of ovulation is a criterion for judging the extent of ovulation. The average number of corpora lutea observed per animal subsequent to the injection of 1.6 C-N units of PMS was 8.80 (Table VII). This number represents an increase of 141.10 percent above that in untreated animals; no other dose gave as great an increase in the number of these bodies. Seventy percent of the animals treated with this dose exhibited an increase in number of 50 percent or more, and of the remaining 30 percent, one animal had four corpora, a second animal had one, and a third had no corpora lutea. Inducing 70 percent of the animals to show an increase in ovulatory response seems satisfactory when a comparison is made with swine and cattle. Heitman and Cole (1956) were able to induce 76 to 86 percent of PMS-treated sows to come into estrus and ovulate; and Hafez (1962) demonstrated that 72 percent of PMS-treated cows exhibited non-behavioral estrus (ovulation). However, when

compared with rats and mice, 70 percent is not a good response. Rowlands (1944) induced 100 percent of PMS-treated rats to ovulate, and Fowler and Edwards (1957) induced estrus in 75 percent with ovulation in 99 percent of PMS-treated mice, regardless of the estrus stage in which they were treated. The only dose of PMS which induced a larger percentage of the animals in this study to exhibit an increase in the number of corpora lutea of ovulation was 16.0 C-N units (Table X). However, with doses of 8.0 and 16.0 C-N units, there was an increase in the incidence of cystic follicles, with egg retention.

A consideration of potential litter size is also important when determining the optimum physiological dose of PMS. Fowler and Edwards (1957) demonstrated that, although some PMS-treated mice gave birth to larger litters, the mean litter size did not increase above normal due to greater resorption and irregular distribution of the embryos in the uterus. Although animals may be induced to ovulate larger numbers of ova with larger numbers of implanted egg cylinders, the physiological and anatomical limitations of the animal will determine the number of young that can be carried alive to term. Therefore, no practical goal is achieved by the use of such superovulation procedures. The largest single litter recorded for the chinchilla, in which all of the babies survived, was nine; a few animals regularly have litters of four to six babies; however, the average litter size for chinchillas is only two (personal communication, Hillemann, Oregon State University; Hillemann, Tibbitts, and Gaynor, 1959). Subsequent to 1.6 C-N units of PMS, 60 percent of the animals treated exhibited a total of 6 to 12 corpora lutea of ovulation. It appears

that the physiological and anatomical capabilities of chinchillas are such that they could carry from 6 to 12 embryos to term.

The results of this study suggest several lines for further investigation. Several investigators have obtained better ovulatory responses when PMS was followed by an injection of HCG or some other luteinizing agent (Rowlands, 1944; Saunders, 1947; Hammond, 1952; Will, 1962; Swyer, 1964; and Wyss and Pincus, 1964). Because of the small number of large follicles in chinchillas it appears that PMS injected by itself is sufficient to induce ovulation. Therefore, it is doubtful that the administration of a luteinizing agent following PMS would significantly increase the ovulatory performance in these animals. The observed increase in the number of small follicles following PMS raises the question about their fate following a second injection of PMS. It is possible that these follicles would be stimulated to accelerate their growth and subsequently ovulate. It is also possible, barring extensive follicular atresia, that these follicles (without the second PMS injection) would ovulate during the next estrus in the measure of a superovulation.

It is desirable to develop a reliable and manageable method to follow the phases of the chinchilla estrous cycle. A variety of techniques, in addition to vaginal smears, have been useful for determining the onset of estrus in other rodents such as rats. These techniques include observing (1) an increase in running and other physical activity, (2) twitching of ears, (3) an increased receptivity to the male, (4) vulval swelling and color changes, and (5) the lordosis response to pudendal stroking. Perhaps some of

these or even other signs may be found to be reliable for chinchilla.

As noted above, although superovulation and increased prenatal litter size may occur, there may also be an associated increase in prenatal death with no net increase in the number of young born. It would be of interest to know whether an increase in the number of babies born would follow superovulation in chinchillas. It is possible that, if an increased number of implantations did occur subsequent to superovulation, the chinchillas physiological capacity would be sufficient to carry an increased number of young to term. Perhaps supplementary progesterone would have to be administered in order to enhance the production of superlitters.

Repeated superovulation may also exhaust the ovary and thereby compromise the reproductive life of the animal. An ovary may also become refractory to ovulation due to the production of anti-PMS serum, or to some other unidentified physiological process.

SUMMARY

1. The object of this study was to investigate the possibilities of inducing superovulation in the chinchilla by establishing the optimum dosage of PMS to produce a maximum ovulatory response, and to compare chinchilla with other animals in this respect.
2. Non-pregnant female chinchillas were given a single subcutaneous injection of 1.0, 1.2, 1.5, 1.6, 1.8, 2.0, 3.3, 4.0, 8.0, or 16.0 C-N units of PMS.
3. The ovaries of these females were examined to determine the effect of PMS on ovarian weight, on the number of small follicles, large follicles, accessory corpora lutea, and corpora lutea of ovulation. The results of this analysis were compared with the information of Hillemann, Tibbitts, and Gaynor (1959) for untreated females.
4. Following PMS treatment an increase was observed in ovarian weight, in the numbers of small follicles, accessory corpora lutea, and corpora lutea of ovulation, but not in the number of large follicles. The higher PMS doses induced also an increase in cystic follicles.
5. In terms of over-all ovarian response, and especially in terms of ovulatory response, it appears that the optimum PMS dose for chinchillas is 1.6 C-N units.
6. From an analysis of the percentage of animals exhibiting an increase in ovulatory response, and of the degree of ovulation in individual animals following PMS, it appears that the best response

is achieved when PMS is given on the follicular phase of the estrous cycle. In addition, anestrous chinchillas apparently cannot be stimulated to ovulate with PMS.

7. A number of projects needing further consideration include establishment of the estrous cycle, the fate of artificially increased number of small follicles, the fate of increased implantations, and the possibility of ovarian exhaustion.

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