FOLLICULAR GROWTH AND STEROIDOGENESIS IN THE NONPREGNANT, PREGNANT AND PROGESTERONE - TREATED NEW ZEALAND WHITE RABBIT

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NEW ZEALAND WHITE RABBIT

This thesis submitted by Swarna Lata Setty has been examined and approved by an appointed committee of the faculty of the School of Graduate Studies of the Medical College of Georgia.

The signatures which appear below verify the fact that all required changes have been incorporated and that the thesis has received final approval with reference to content, form and accuracy of presentation.

This thesis is therefore accepted in partial fulfillment of the requirements for the degree of Master of Science.

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DEDICATION

This manuscript is dedicated to my father, Dr. S.V. Satyanarayana Setty, and my mother, Premaleela Setty; their love, support and encouragement inspired the faith necessary to initiate and complete this degree. Thank you!

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INTRODUCTION

General Introduction and Statement of Problem

In the female mammal, the primary function of the ovary consists of the formation, differentiation, maturation and delivery of it's germ cells, the oocytes. Shortly after birth, the mammalian ovary contains it's full complement of oocytes but the growth of each oocyte is arrested at the immature, dictyate stage of meiosis. This inhibition of growth is maintained until oocyte maturation resumes in the preovulatory follicles, in response to the stimuli presented in the preovulatory gonadotropic surges (Ganong, 1983). The mechanisms that initiate the resumption of growth of a single egg, in the case of monotocous species, or a few eggs, in the case of polytocous species, are decidedly complex. All the follicles are exposed to the same concentrations, as well as fluctuations, of serum gonadotropins but only a few are recruited into the growing phase. Once the oocytes resume their progression towards maturation, the greater proportion spontaneously become atretic, degenerate and disappear. The remaining small number of follicles containing mature oocytes are ovulated and thereby attain their gametogenic potential (Goldfien and Monroe, 1983). The ability of the follicle to complete ovulation followed by proper embryonic development is dependent upon a long, complicated process of events. This sequential series of events not only leads to the maturation and differentiation of the oocyte, but also to the development of the follicle (Genuth, 1983). Furthermore, the thecal and granulosa cells of the follicle are responsible for providing the appropriate

microenvironment by generating the proper hormonal milieu necessary to ensure the oocyte's growth and development. The maintenance of fertility in the female is dependent upon the establishment of the correct intrafollicular environment in which to ensure the fertilizable status of the ovum. Therefore, ovarian follicular growth and development play a crucial role in the reproductive process of the female mammal.

While follicular development is a highly ordered process regulated by both intra-ovarian and extra-ovarian factors, the exact regulatory mechanisms have not yet been fully elucidated. Nonproliferating primordial follicles are formed during fetal development with each oocyte being surrounded by a single layer of flattened epithelial cells. Primordial follicles are principally found in the peripheral cortex of the ovary, but as these early follicles begin to develop they migrate deep into the ovary, thereby forming a distinct medullary zone. Follicles destined to ovulate are recruited from these primordial follicles and stimulated to grow and mature by pituitary gonadotropins. The growth of these follicles can be divided into two distinct stages relative to the growth of the oocyte (Brambell, 1956). In the first stage, the oocyte grows rapidly towards it's maximum size while the follicle size increases gradually. The second stage consists of rapid growth of the follicle while not much change is visible in the size of the oocyte. The final size of the follicle, after completion of it's rapidly proliferative phase, is directly proportional to the animal's body size (Parkes, 1931). As the ovarian follicle grows, it is surrounded by an envelope of condensed connective tissue derived

from the stromal matrix and organized to form a highly vascularized internal layer, the theca interna. An external fibrous layer, the theca externa, is continuous with the tunica albuginea, a dense white, fibrous sheath covering the entire follicle. As the theca continues to thicken by hyperplasia and the addition of more cells from the stroma, vascular loops are formed that cover the entire surface of the follicle. The granulosa cell layer remains avascular until after ovulation due to the presence of a basement membrane which separates the granulosa cell layer from the thecal cell layer. Concomitant with the formation of the basement membrane, there is a rapid increase in the mitotic activity of the granulosa cells resulting in the ovum being situated in the center of a solid ball of cells called the membrana granulosa. As the follicle matures due to the rapid proliferation of the granulosa and thecal cells, small fluid filled sacs are formed in the membrana granulosa. These antra eventually coalesce to form one single, large antrum within which the ovum is suspended. The ovum, located in the fluid filled antral cavity, is surrounded by an irregular group of granulosa cells, the corona radiata, which anchors the ovum to the follicular wall.

The importance of the pituitary gonadotropins, follicle stimulating hormone (FSH) and luteinizing hormone (LH), in supporting the initiation and progression of follicular maturation throughout the reproductive life of the female has been well investigated (Finding and Tyrrell, 1983). However, of the follicles present in the ovary at birth, the vast majority are destined to be lost to atresia. Throughout the various stages of

development, only a few exceptional follicles, derived from a cohort of growing follicles, escape atresia and advance to ovulation. This selection process has not yet been fully elucidated but the number that undergo ovulation is usually characteristic for each individual species. Furthermore, the mechanism regulating the ovulatory quota has not yet been clearly defined but, in most mammalian species, approximately the same number of follicles ovulate during each reproductive cycle. FSH and LH are known to exert tremendous influence before puberty, during puberty and throughout the adult reproductive life of the female mammal. These polypeptide hormones, along with other hypothalamic and pituitary factors, have the greatest responsibility in controlling the differentiation and ovulation of the follicle and formation of it's successor, the corpus luteum. These same factors are also active in establishing the proper hormonal milieu to maintain the ovum throughout fertilization and it's early embryonic development. The entire female reproductive cycle revolves around the proper concentrations and pulsations of gonadotropin secretion and the positive and negative feedback modulation of the hypothalamic-pituitary-ovarian axis.

Previously, follicular growth and maturation in the normal, intact female mammal had been completely attributed to the gonadotropic secretions originating from the anterior pituitary gland. However, more recent observations have indicated that along with FSH and LH, ovarian factors may be influencing the follicular maturational process. For example, after a normal ovulation, there seems to be a natural hiatus in follicular

growth during the luteal phase of the ovarian cycle in primates (Goodman, 1977) and in humans (Ross, 1976). Furthermore, the FSH surge occuring immediately after ovulation in the rabbit, was thought to initiate a wave of new follicle growth. These new follicles were throught to be necessary for pregnancy because they secrete the luteotropic estrogen essential in maintaining normal luteal function, since the corpoa lutea become estrogen dependent on day 6 post coitum (p.c.). However, when this postovulatory FSH surge was blocked, pregnancy continued. This indicated that the growth of follicles continued despite the absence of FSH release.

In considering factors which regulate follicular growth and development, an interesting phenomena has to do with follicle development which occurs during periods of high circulating concentrations of progesterone. During these periods, (luteal phase, pregnancy, pseudopregnancy) the steroid would be expected to suppress circulating levels of gonadotropins and thereby reduce follicle growth. This seems to be the case in many published studies. For example, in many mammals, the majority of follicles present in the ovary during periods of elevated progesterone are found to be atretic. Bogovich et al. (1981) noted that during late pregnancy in the rat, a time during which progesterone levels are decreasing, there is an increase in FSH levels and of a new wave of follicle growth is initiated. DiZerega et al. (1979) found that although follicular growth is absent in the ovaries of the pregnant monkey, likely due to the suppression of gonadotropin secretion by the high levels of circulating

progesterone, the pregnant monkey ovary remains completely responsive to gonadotropin stimulation. It would seem that, in humans (Baird, 1984) and other primates, the inhibitory action of progesterone on follicular growth is mediated by the suppression of gonadotropin secretion. However, other investigators have found that luteectomy, (corpus luteum ablation), performed either in the luteal phase or during pregnancy, is followed by a wave of new follicle growth without any change in serum levels of FSH (DiZerega, 1981).

Progesterone may not only be acting indirectly to inhibit follicle growth through the suppression of gonadotropin secretion, but may also regulate follicular development directly. Nillson et al. (1982) reported that when luteectomy was performed in the luteal phase of Rhesus monkeys, the dominant follicle of the next cycle was found on the same ovary. When Koering et al. (1982) removed an actively secreting corpus luteum from a luteal phase monkey ovary, after 4 days there was a greater number of follicles in the ovary which did not contain the original corpus luteum. Similarly, Hodgen and DiZerega, (1981) discovered an inverse relationship between follicle growth and progesterone content in the monkey ovary, with the ovary secreting the greatest amount of progesterone containing the fewest number of follicles. Furthermore, Baird (1984) found that in the human ovary containing an actively secreting corpus luteum, almost all the large follicles were atretic. These experiments can be utilized support for a role of luteal progesterone in inhibiting adjacent follicle development.

In contrast, Dufour and coworkers (1971) found that, in

sheep, more follicular growth occurred in the presence of active corpora lutea. Using the same species, Cahill and coworkers (1981) reported a positive correlation between serum concentrations of progesterone and the number of follicles in the ovary. In these instances, progesterone may be exerting a stimulatory action on ovine follicular growth and development.

In another study, Mills and Gerardot (1984) investigated the association between copulation and ovulation in the pregnant rabbit. These investigators showed that ovaries of pregnant rabbits respond to injections of hCG or LHRH by ovulating the estrogen producing follicles. This resulted in the failure of the estrogen dependent corpora lutea and to the termination of the existing pregnancies. The LHRH injections also stimulated LH release, demonstrating that the pituitary remains responsive to LHRH. However, in pregnant rabbits, copulation failed to induce ovulation and therefore did not terminate pregnancies. In order to determine if the high concentrations of progesterone present during pregnancy were exerting a protective action on the pregnancy, nonpregnant rabbits were treated with progesterone and then mated or injected with hCG or LHRH. In the progesterone treated-nonpregnant rabbits, hCG and LHRH injections caused ovulation while mating did not. It was concluded that progesterone had somehow disconnected copulation from the release of LH and FSH by the pituitary. This study proves that follicles capable of ovulation are present throughout pregnancy but are protected against ovulation by a progesterone-mediated dissociation of hypothalamic LHRH secretion (Mills and Gerardot, 1984).

All these lines of evidence point to the interesting possibility that besides LH and FSH, progesterone may be a principal regulator of follicular growth and function in the mammalian ovary. In order to further investigate the action of this steroid hormone on folliculogenesis, the rabbits were employed in the experiments presented in this proposal.

The rabbit is an induced ovulator and coital stimulation triggers neuroendocrine reflex mechanisms which release the pituitary gonadotropins, luteinizing hormone and follicle stimulating hormone. In the absence of mating, rabbits demonstrate an estrous period that may continue for several months. Early investigators had thought that mature follicles are present more-or-less constantly throughout this estrous period, with the formation of blood engorged follicles marking follicular regression (Heape, 1905). However, direct observations show that follicular growth and turnover occurs continuously. Hill and White reported that mature follicles grow and regress during the estrous period. They estimated that follicles survive from 7 to 10 days (1933). However, DeSaive reported the time necessary for small, pre-antral follicles to grow to ovulable size was approximately 18 days (1949). In studying follicular growth during pregnancy, Adams reported follicles of ovulable size during various stages of rabbit pregnancy which ovulated in response to injections of human chorionic gonadotropin (hCG) (1968). Asami showed that not only were large follicles present within 2 days after ovulation, but even a greater number of these large follicles were present by 7 days following ovulation (1920). Previous studies

done in this laboratory indicated that very few large follicles were present in ovaries collected 48 hours after mating, since most of the mature follicles were lost to ovulation. However, within the next 4 days, there was significant follicular growth with greater than 30% of the follicles on day 6 being of ovulatory size (Osteen and Mills, 1980). The new follicles were also steroidogenically active with elevated content of estradiol and testosterone.

Accordingly, the ovaries of pregnant rabbits contain not only many actively secreting corpora lutea, but also numerous Graafian follicles. The growth and maturation of these follicles is extremely important since these follicles produce the estrogen necessary for the maintenance of the corpora lutea, and therefore, pregnancy (Keyes and Nalbandov, 1967). Both Adams and Stormshak (1968) and Casida (1964) discovered that by injecting a standard dose of hCG or LHRH, ovulation could be induced throughout the various stages of pregnancy. This indicates the existence of ovulable size follicles throughout the duration of pregnancy.

However, in contrast to follicles from non-pregnant, intact animals, follicles from pregnant animals are functioning in an environment saturated with progesterone. Although folliculogenesis has been studied extensively in the non-pregnant mammal, the growth and turnover of follicles in the pregnant mammal has not yet been fully investigated. Bahr and Shahabi et al. (1979) studied adenyl cyclase activity of follicles and revealed that follicles obtained from estrous, non-pregnant rabbits displayed

greater adenylate cyclase activity than follicles from pregnant and pseudopregnant rabbits. Adenyl cyclase, activated by the formation of the hormone-receptor complex, provides an indirect measurement of receptor binding. This enzyme also catalyzes the conversion of ATP to c-AMP, the intracellular secondary messenger of polypeptide hormones. These investigators found a positive correlation between follicular adenylate cyclase activities and the steroidogenic capabilities of follicles. Interestingly, Bahr and Shahabi et al. discovered that there was a progressive decrease in follicular responsiveness to LH and FSH with increasing length of pregnancy. Therefore, the greater the duration of time that follicles are located in close proximity to the corpora lutea, the less estrogen and testosterone were secreted in response to gonadotropic stimulation. These investigators proposed that the steroid synthetic capacity of a follicle is regulated not only by circulating gonadotropins, but may also be influenced by the intragonadal level of steroids. Richards and Midgley (1976) reported that estrogen, secreted by follicles under FSH stimulation, exerted a regulatory role in follicular growth through the induction of LH receptors. Furthermore, other investigators (Schomberg, Stouffer, et al. 1976; Lucky, Schreiber, et al. 1977) provided information on testosterone, which when added to <u>in vitro</u> systems, caused increased production of progesterone by granulosa cells. These results are examples of gonadal steroids exerting a positive effect on ovarian function. It is also plausible that certain steroids may exert a negative influence on ovarian activity as well.

The preliminary results of Bahr and Shahabi (1979) suggest a possibility that high intragonadal concentrations of progesterone, such as that found during pregnancy, may depress follicular steroid secretion.

The effects of elevated levels of progesterone on ovarian function are being more fully investigated. Harper (1962) found that exogenous progesterone prevented the ovulation that usually occurs 10-12 hours following copulation. Hisaw (1947) stated that an actively secreting corpus luteum prevented ovulation, not by hindering follicular growth, but rather by prohibiting the rupture of the follicle. However, Nillson et al (1982) found that following the removal of an actively secreting corpus luteum, the next dominant follicle usually appeared on the ipsilateral ovary. Furthermore, Hodgen and DiZerega (1981) reported that the monkey ovary producing the most progesterone usually also contained the fewest follicles. This inhibitory action of the corpus luteum seems to be reversible. In the monkey ovary, removing an actively secreting corpus luteum during the luteal phase, resulted in more follicles being found on the contralateral ovary 4 days later (Koering et al. (1982). This contention was supported by the observation of Baird et al. (1984), that in the human ovary, almost all the large follicles found in the luteal phase were atretic. It would seem that when a corpus luteum is actively synthesizing and releasing progesterone, adjacent follicular growth is suppressed. Administration of exogenous progesterone also inhibited the

growth of follicles, and therefore prevented ovulation (Rothchild, 1965). Wallach and Noriega (1970), elevated circulating progesterone utilizing progesterone implants and found an increase in the percentage of early, preantral follicles, but a decrease in the number of mature, antral follicles. Finally, Bahr and Gardner et al. (1980) studied the effect of high concentrations of progesterone, on follicular steroidogenesis. In these studies, follicles were isolated from pregnant, pseudopregnant and non-pregnant rabbits, placed in perfusion chambers and steroid synthesis stimulated with various hormones. These investigators reported that follicles isolated from pregnant and pseudopregnant rabbits exhibit different patterns of basal and gonadotropin stimulated secretion of estrogen and testosterone. In follicles removed from pregnant and pseudopregnant animals, there was a decrease in gonadotropin stimulated secretion of estrogen and testosterone. Follicles obtained from non-pregnant animals incubated with various concentrations of progesterone displayed a depressed basal and LH-stimulated secretion of estrogen. However, follicles obtained from control rabbits, after being stimulated by the same dosage of LH, showed a 2-fold increase in estrogen secretion. This study suggests that elevated levels of progesterone may exert a negative effect on follicular steroid production. This contention is strengthened by Moor and Walters (1979), who discovered that after culturing follicles with luteal tissue, the morphological structures as well as the steroid producing capabilities of follicles were

modified. Taken together, these studies suggest that elevated levels of progesterone exert a regulatory role in follicle growth and steroid production.

Based on the previous discussion, it is clear that follicular growth is ongoing during pregnancy. However, it is not clear what factors are regulating this growth since both LH and FSH may remain at low, basal levels throughout the entire gestational period. It is therefore proposed that progesterone, via a direct action on the follicular growth process, is the principle regulator of the development of follicles in the pregnant rabbit ovary. The following investigation was designed to confirm the extent of follicular growth during periods of high circulating progesterone and to examine the role of progesterone in this growth.

REVIEW OF RELATED LITERATURE

Hormonal Control of Ovarian Follicle Development

Ovarian follicles are complex structures that undergo continuous assembly, growth and regression. This turnover of follicles continues, irrespective of the physiological status of the animal, including pregnancy, lactation and other interruptions of cyclicity which occur during the reproductive life of the adult animal (Peterson and Peters, 1971). This constant follicular growth occurs in both spontaneously and reflexly ovulating animals. The dynamic phenomena of the maturational processes of ovarian follicular growth was discovered as early as 1672, by Reinier de Graff (Catchpole, 1940), and centuries later by the noted histological studies of Hill and White (1933). Although it had been known that both intraovarian and extraovarian mechanisms regulate follicular development, it was only within the past decade that significant progress has been made in determining the factors which ultimately decide the fate, ovulation or atresia, of each follicle (Peters et al. 1975; Lindner et al. 1977; Edwards et al. 1977). Follicular development has been divided into arbitrary stages based on general morphological characters, such as the absence or presence of an atrum, follicular diameter and number of cell layers which comprise the follicle (Pedersen and Peters, 1968; Mariana, 1972; Nicosia, 1974). Follicle growth has also been separated into gonadotropin-independent (primordial) and gonadotropin-dependent (preantral and antral) stages of development. Peters et al. (10) derived support for non-

gonadotropic regulation of folliculogenesis from their study that indicated follicular fluid factors may regulate the initiation of follicle growth, as well as the rate by which follicles leave the pool of non-proliferating follicles (Peters, 1978). Furthermore, cohorts of primordial follicles in prepubertal girls, in whom LH and FSH levels were undetectable, and in hypophysectomized lab animals are constantly leaving the large pool of inactive primordial follicles and resuming growth (Goldfien and Monroe, 1981). Nevertheless, a complete lack of gonadotropic support may not be effective, and gonadotropins, particularly FSH, may still be required for the normal morphological differentiation and organization of follicular cells and perifollicular structures (Eshkol and Lunenfeld, 1972). This contention is strengthened by the finding that FSH receptors are present on the granulosa cells of primordial follicles (Richards et al. 1976).

Development of follicles beyond the preantral stages depends upon the presence of the pituitary gonadotropic hormones. In their pioneering studies, Greep et al (1942) demonstrated the secretion of two separate gonadotropins, FSH and LH, by the pituitary gland. Other investigators found that whereas neither FSH nor LH alone was able to stimulate follicular maturation, a combination of FSH and LH stimulated estradiol synthesis, follicular maturation, ovulation and luteinization (Lostroh and Johnson, 1966). However, the individual roles of each polypeptide hormone in regulating folliculogenesis have not yet been fully defined.

Progress has now been made in identifying the individual functions of FSH and LH, as relating to follicular maturation and steroidogenesis. FSH, as indicated by it's name, is shown to play a primary role in maintaining ovarian follicular development. During follicular development, granulosa cells divide and acquire FSH receptors. By utilizing autoradiographic binding techniques, it has been revealed that these FSH receptors are localized exclusively on the granulosa cells of follicles (Midgley, 1973). These FSH receptors seem to be present throughout the various stages of follicular growth, thereby indicating that these binding sites are a constitutive component of the granulosa cell plasma membrane. Furthermore, it has been demonstrated that FSH binding sites are retained in granulosa cells of hypophysectomized rats and that even after hypophysectomy, FSH can still stimulate granulosa cell accumulation of cAMP (Richards et al. 1979), increased cAMP binding sites (Richards and Rolfes, 1980) and increased FSH receptor content (Lindner et al. 1974; Ireland and Richards, 1978). In response to FSH, these granulosa cells acquire enzymes essential for steroid biosynthesis, and also acquire cell surface receptors for LH (Erickson and Hsueh, 1979).

Luteinizing hormone plays a principal role in stimulating ovulation and luteinization. In addition to the preovulatory LH surge, small but sustained increases in basal concentrations of LH play a critical role in determining which follicles will enter the final stages of preovulatory growth (Carson et al. 1981; Karsch, 1980). Autoradiographic and binding analyses have demonstrated that LH receptors are an integral part of all theca cells but

appear only on granulosa cells found on developing preovulatory follicles (Channing and Kammerman, 1974). These observations indicate that although LH may only act on theca cells of small follicles, this hormone acts on both theca and granulosa cells of large follicles prior to ovulation. Therefore, the role of LH in follicular cell function prior to the LH surge is obligatory for proper follicle growth. Immediately prior to ovulation, exposure to the preovulatory LH surge brings about the resumption of growth and meiotic maturation in the oocytes within the preovulatory follicles. These immature oocytes complete maturation up to the first polar body stage (Channing and Tsafriri, 1977). Moreover, LH stimulates the granulosa cells to increase the number of LH/hCG receptors by a 100-fold. The cells increase in their ability to respond to LH by accumulation of cAMP (Lindsay and Channing, 1978). In addition, the granulosa cells luteinize morphologically and acquire the ability to secrete progesterone, when they are removed from the follicle and cultured (Channing, 1970; Channing, 1970). As a group, these experimental findings point to the absolute necessity of both LH and FSH for the initiation of follicular growth, ovulation and luteinization (Richards, 1980).

Maintenance of Corpora Lutea Function

The importance of progesterone in the implantation of the fetus and the maintenance of pregnancy has been well documented. Early investigators such as Allen and Corner (1929) have provided evidence that castration during early pregnancy prevented embryonic development and implantation. However, application of corpora lutea extracts to rabbits castrated 24 hrs after mating resulted in continuation of pregnancy. The same results were obtained by Wu and Allen (1958) when they applied progesterone or 17-alpha hydroxyprogesterone caproate to rabbits castrated 24 hrs after mating. This indicates that administration of exogenous progesterone maintains pregnancy in the castrated animal. Therefore, progesterone, secreted by the rabbit corpus luteum, is the absolutely essential progestational agent required for the sustenance of pregnancy.

Although corpora lutea were known to synthesize and secrete progesterone, the exact mechanism by which this was done had not yet been fully defined. It was known that if hypophysectomy was performed at any time during pregnancy or pseudopregnancy, the corpora lutea promptly regressed along with a rapid loss of any progestational effects found in the uterine horns. LH was thought to be the hypophyseal luteotrophin, as this hormone had already been shown to maintain actively secreting corpora lutea in hypophysectomized pseudopregnant rabbits (Kilpatrick, Armstrong, et al. 1964). These same investigators also showed that prolactin was not capable of maintaining the corpora lutea, and was therefore not a luteotropin in the rabbit.

Keyes and Nalbandov (1967) presented data indicating that when ovarian follicles were destroyed by X-irradiation, but the corpora lutea and interstitial tissue were left intact, not enough progesterone was being produced to maintain pregnancy. However, if 2 to 4 μg of estradiol was administered to pregnant animals with ovaries which contained only corpora lutea and interstitial tissue, pregnancy was maintained. Progesterone was present in both the corpora lutea and ovarian effluent plasma. Neither LH nor whole anterior pituitary powder were able to maintain the corpora lutea or stimulate it to secrete progesterone in these animals. Furthermore, LH could not sustain pregnancy in the absence of estrogen. All these lines of evidence support the contention that estrogen, and not LH, maintains functional corpora lutea and pregnancy. Keyes (1969) concluded that the actions of both LH and 17β-estradiol are necessary; LH to initiate luteinization of the Graafian follicle and stimulate estrogen synthesis and 17-β-estradiol to maintain the corpus luteum.

Younglai (1972) measured follicular fluid estradiol concentrations and noted that following the preovulatory peak, estradiol fell to very low levels prior to ovulation. Estradiol remains at this basal level until three days after ovulation, although the corpora lutea are being formed during the same time period (Hilliard and Eaton, 1971). Ectopic corpora lutea which were allowed to develop in ovariectomized animals, four days post coitum appeared to be histologically normal (Keyes and Armstrong, 1968). All these studies suggest that estrogen may

not be necessary for the luteinization and early development of the corpus luteum. Furthermore, Miller and Keyes (1975) demonstrated that if estrogen is not present by day 5 post coitum, luteal regression occurs and the tissue dies. These investigators showed that the corpora lutea does not display estrogen-dependency until 5 to 6 days after mating, but that at this critical time, the presence of estrogen is absolutely essential for continued luteal function. Concomitant with this transition to estrogen-dependency by the corpora lutea, is the appearance of cytosol receptors which display a high affinity for estradiol (Mills and Osteen, 1977). Miller and Keyes (1978) noted that from day 3 to day 6 of pseudopregnancy, there was almost a 400% increase in the amount of estradiol bound to the high affinity cytosol receptor sites. They also found that around day 5 post coitum, the cytosol receptors, after binding to estradiol, acquire the capacity to be translocated into the nucleus. Day 5 post coitum, the day of highest estrogen binding, indicates the transition of the corpus luteum to estrogen dependency. In the rabbit, the luteotropic role of estrogen is essential for the maintenance of pregnancy because, in this species, the placenta is incapable of producing progesterone. In the presence of viable fetuses and placentae, the corpora lutea of pregnancy stay responsive to estrogen throughout the gestational period. However, earlier observations had indicated that during the second half of gestation (day 22) a luteotropic factor might be secreted by the fetal placenta (Gadsby and Keyes, 1982). However, when estrogen was withdrawan, after day 22, there

was a rapid decline of serum progesterone levels resulting in spontaneous abortions. If indeed, the placenta is playing a luteotropic role in maintaining the corpora lutea of pregnancy, the role is expressed only if estrogen is present. Estrogen will not maintain the corpora lutea of pregnancy if fetuses and placentae are removed. However, if fetuses and placentae are left intact and estrogen is withdrawan, pregnancy still fails. These findings show that, both estrogen and the fetal placentae need to be present throughout the gestational period and these luteotropic agents probably work in conjunction with each other to maintain the corpora lutea of pregnancy (Gadsby, Keyes, Bill, 1983).

MATERIALS AND METHODS

Animals

Sexually mature female rabbits of the New Zealand White strain were purchased from a local supplier and housed in separate cages with water and rabbit chow provided ad libitum. The rabbits were kept at constant temperature and maintained on a 14-hour light, 10-hour dark, lighting regimen.

In most studies, 48-72 hrs before induction of ovulation does were injected with 50 IU pregnant mare serum gonadotropin (PMSG). In some experiments, does were mated by introducing them into the cage of a vigorous buck rabbit. Successful matings were confirmed by microscopal examination for the presence of viable spermatozoa in vaginal washings obtained immediately after mating. In experiments which utilized progesterone-treated does, elevation of circulating progesterone was accomplished by implanting pellets of pure progesterone. Control animals (day 0) consisted of intact, nonpregnant animals which did not receive any type of treatment. All animals were sacrificed by cervical dislocation.

Formation of Progesterone Pellets

To prepare the progesterone pellets, 50 mg of crystalline progesterone was placed on the platform of a hand press generally used for the preparation of sodium bromide pellets to be analyzed by infrared spectroscopy. With application of pressure for 30 seconds, pellets of 1 mm in thickness and 7 mm in diameter could be produced. In preliminary experiments, it was

determined that 8 pellets placed in separate areas in the dorsal neck region of rabbits via 2 incision points (to insure proper exposure of the total surface area of the pellets for maximum delivery rate of the hormone) were sufficient to elevate serum progesterone levels to about 5-8 ng/ml, close to the levels measured in pregnant animals.

Implantation of Progesterone Pellets

Each intact, control rabbit was implanted with eight progesterone pellets. Prior to implantation, each animal was anesthetized with injections of Ketamine (3.0 ml/kg body weight) and Xylazine (1.5 ml/kg body weight). The back of the neck was shaved employing an electric razor and the shaved area was cleansed with alcohol. Then, two separate incisions were made in the shaved area, and four progesterone pellets were implanted at each incision point. The wounds were closed with wound clips and the entire nape was saturated with alcohol to prevent any infection.

Buffers

Steroid radioimmunoassays were performed in phosphate gel buffer (PGB) which contained 150 mM NaCl, 39 mM $NaHPO_4$, 61 mM $NaHPO_4$ and 0.1% gelatin. The pH was adjusted to 7.0 by the addition of 1N NaOH.

Gonadotropin radioimmunoassays were performed utilizing phosphate buffered saline (PBS) composed of 0.14 M NaCl and 0.01 M $NaPO_4$ added as a dilution of 0.5 M NaH_2PO_4 and 0.5 M Na_2HPO_4. The pH was adjusted to 7.5 with 1N NaOH.

Follicle Collection

In all the experiments conducted, ovaries were removed from sacrificed rabbits via an abdominal incision. The ovaries were immediately placed in ice cold saline and transported to the laboratory. Under 7X magnification, follicles $\geq$ 1 mm were freed of adhering interstitial tissue according to the method of Mills et al. (1971). The external diameters of individual follicles were then measured utilizing a calibrated ocular micrometer with subsequent conversion of these values to millimeters. Follicles were then placed in individual test tubes containing 500 μl of distilled water and frozen at -20^{o}C for subsequent analysis of steroids by RIA.

Extraction of Steroids from Whole Follicles

In order to determine the steroid content of the individual follicles collected by the procedures outlined in the previous section, each follicle was ruptured by pressing it against the wall of a test tube with a long glass rod. The steroids were extracted by adding 10 ml of ether, the resulting solution was then mixed by vortexing for 2 minutes and the aqueous and ether phases separated by freezing in an acetone/dry ice bath. The aqueous phase was frozen while the ether phase containing the dissolved steroid did not freeze and was decanted. The ether was evaporated with a nitrogenous stream and the steroid containing residue reconstituted in 2.5 ml phosphate-gel buffer for assay of steroid content by RIA.

Extraction of Steroid from Serum

Blood samples were collected by direct cardiac puncture from rabbits that were "hypnotized" by constant rubbing around the eyes and muzzle area. The blood was allowed to clot in the refrigerator for 24 hours. The samples were then centrifuged at 4 °C for 30 minutes at 2000 rpm and the resulting sera aspirated and kept frozen at -20 °C until assayed. 100 µl aliquots of sera were extracted with 10 ml of ether and the resulting steroid containing extract reconstitued in 1.6 ml of phosphate-gel buffer for assay of steroid content.

Steroid Radioimmunoassay

Steroids

Radioactive steroids were obtained from the New England Nuclear Corporation in Boston, Massachussetts and used in the measurement of serum steroid levels by radioimmunoassay. The steroids purchased were: {2,4,6,7-^{3}H} estradiol-17β (Specific Activity 111.0 Ci/mmol), {1,2,6,7-^{3}H} testosterone (Specific Activity 91.1 Ci/mmol), and {1,2,6,7-^{3}H} progesterone (Specific Activity 90.1 Ci/mmol). Purity of radioactive steroids was checked prior to use by column chromatography and further purified if necessary. All non-radioactive steroids were purchased from Schwarz/Mann.

Steroid Antibodies

The antiserum to estradiol (E_2TGK) was a gift from Drs. D.C. Collins and Kristina Wright of Emory University, Atlanta, Georgia. The E TGK antiserum was raised against estradiol-6-(o-carboxymethyl) oxime-thyroglobulin and was utilized at a dilution of 1:40. The antiserum to testosterone (X-181) was obtained from Radioassay Systems Laboratories, Inc., Carson, California. The X-181 antiserum was raised against testosterone 19-carboxymethyl ether - bovine serum albumin (BSA) and was used at a dilution of 1:25. The antiserum to progesterone (D_3) was prepared in the laboratory of Dr. V.B. Mahesh, Medical College of Georgia, Augusta, Georgia. The D_3 antiserum was prepared in rabbits against 4-Pregnen-11β-ol-3, 20-dione hemisuccinate - BSA. The D_3antibody was used at a final dilution of 1:60.

Dextran Coated Charcoal

In order to separate the bound steroid in the incubation medium, Dextran coated charcoal is added to precipitate out the unbound steroid. This charcoal preparation contained 2.5 gram Norit "A" charcoal and 1.0 g dextran which had previously undergone several washes with distilled water to remove "fines" and then was suspended in phosphate-buffered gel buffer prior to use.

Steroid Radioimmunoassay

The radioimmunoassay (RIA) method employed in measuring the steroid content of individual follicles and serum samples was that of Abraham (1971) as modified by Parker (1975) and as previously reported (Mills, 1975; Waterston and Mills, 1976; Mills and Osteen, 1977). The specific antibodies utilized for these steroid assays were E K for estrogen, X-181 for testosterone, and D3 for progesterone. These highly specific antibodies allowed direct assays to be run on extracted samples without need for column chromatographic separation of the individual steroids.

Reference standards, prepared in duplicate, were reconstituted in phosphate-gel buffer (PGB) from steroid concentrations ranging from 5 pg per 0.5 ml to 1000 pg per 0.5 ml for estradiol, testosterone and progesterone. Quadruplicate "total" tubes contained only buffer and ^{3}H steroid while quadruplicate "nonspecific" binding" tubes contained buffer, ^{3}H steroid and dextran coated charcoal (DCC). Unknown samples were assayed in identical fashion as the

standard curve. The antibody was added in 100 µl of buffer, tubes were vortexed and allowed to incubate for 1 hr at 4°C. Approximately 10,000 cpm of the appropriate ^{3}H steroid was next added in 100 µl of PBG buffer and the incubation mixture vortexed. Following another hour of incubation at 4°C, 800 µl DCC was added to adsorb all the unbound steroid. All tubes were mixed and incubated for 10 minutes and then centrifugated at 3000 rpm for 10 minutes at 4°C in order to separate the bound from the unbound steroid. The resulting supernatant, containing the protein-bound steroid, was decanted into 20 ml glass scintillation vials for measurement of radioactivity. The radioactivity was quantified in 10 ml of scintillation fluid (5 g Permablend II (Packard) per liter toluene) by counting in a Beckman LS-7500 liquid scintillation spectrometer. The recorded data obtained from the scintillation counter was then analyzed with a Hewlett-Packard Model 9830-A desk top computer which generated a logit transformation of the standard lines. The unknown steroid concentrations of the experimental samples could then be quantified in reference to the known lines.

Gonadotropin Assay

Gonadotropins

Rabbit serum LH and FSH were measured by utilizing specific homologous double antibody radioimmunoassay kits obtained from the National Hormone and Pituitary Program. These kits contained purified FSH standard (NIAMDD-FSH-AFP-538-C) and purified LH standard (NIAMDD-LH-AFP-559-B). The purified gonadotropins

were radiolabelled with ^{125}I following the protocol of Greenwood et al. (1963) as modified by Osteen and Mills (1979). The purified LH and FSH standards were also diluted for construction of standard curves.

Gonadotropin Antibodies

Antibodies against rabbit FSH (AFP-4-7-21-76) and against rabbit LH (AFP-559-B) had been raised in guinea pigs and were supplied with the kits. The antibody against FSH was diluted to a final dilution of 1:300,000 while the antibody against LH was diluted to a final dilution of 1:3,000,000. In both FSH and LH radioimmunoassays, the hormone-antibody complex was precipitated with the addition of a second antibody, anti-guinea pig gamma globulin, which was raised in our laboratory. The second antibody was used at a dilution of 1:40 for FSH and 1:30 for the LH assay.

Gonadotropin Assay

A standard line was prepared from the purified gonadotropin for each assay. Reference standards were prepared in phosphate buffered saline (PBS), with aliquots of this preparation pipetted in triplicate to give final concentrations ranging from 0.0625 ng/tube to 6.25 ng/tube. These assay standard lines were constructed to establish a reference upon which to calculate the unknown values.

The gonadotropin RIA protocol utilized 200 µl aliquots of serum samples (or standards) pipetted into duplicate, 12 X 75 mm glass test tubes and diluted with 300 µl of 0.1% BSA-PBS to bring

the assay volume to 500 µl. Then, 100 µl of the first antibody diluted in 1:200 normal guinea pig serum was added, with a subsequent 24 hour incubation at 4°C. Next, the ^{125}I labelled gonadotropin (FSH or LH) was added in 100 µl PBS. Following an additional 24 hour incubation period, the second antibody was added in 200 µl of PBS. On the following day, the hormone-antibody complex was isolated by centrifugation, the supernatant aspirated, and the remaining pellet counted for ^{125}I content in the Beckman Gamma Model 8000 Counter.

RESULTS

Characterization of the Size Distribution of Ovarian Follicles During Pregnancy

In the rabbit, estradiol has been recognized as the essential luteotropic hormone, and will maintain functional corpora lutea after hypophysectomy (Robson, 1937; Hilliard, Spies, et al. 1969). The rabbit corpus luteum lacks aromatase activity (Elbaum and Keyes, 1976) and is therefore incapable of synthesizing estrogen. In this species, the ovarian follicles are the sole source of estradiol (Westman, 1934; Keyes and Nalbandov, 1967; Rennie, 1968). Consequently, the growth and development of follicles during pregnancy is extremely important for the maintenance of a normal pregnancy. However, follicle growth and turnover during pregnancy has not been fully investigated. Accordingly, an initial study was completed to examine follicle populations at several times through the gestational period of the pregnant rabbit.

Day 0 (pre-ovulatory, non-pregnant), and Days 7, 14, 21 and 28 of pregnancy, all follicles of diameters $\geq$ 1.0 mm were isolated, freed from adhering interstitial tissue, dissected out, under 7X magnification. Utilizing an ocular micrometer, the external diameters of individual follicles were measured and recorded.

The distribution of sizes of follicles equal to or greater than 1.0 mm in diameter in rabbit ovaries at 0, 1, 2, 3, 4 weeks of pregnancy is presented in Figure 1. These results reveal that by comparing week 0 (non-pregnant) to week 1 of pregnancy, many large follicles (> 1.7 mm) were lost due to ovulation. Throughout the remainder of the gestation period, (Weeks 2, 3, and 4),

although follicles ranging from 1.0 mm to 1.7 mm are continously present, the very large follicles (> 1.7 mm) could not be found.

Figure 2 is a diagramatic representation of the number of follicles of various sizes present during pregnancy. The figure allows the comparison of the frequency of follicles at different stages of pregnancy (Weeks 1, 2, 3 and 4) to the number of follicles present in the non-pregnant animals (week 0). Figure 2 demonstrates that the large follicles (> 1.7 mm) clearly present in the non-pregnant animals (week 0) are not found in the pregnant animals. The absence of large follicles continues throughout the entire gestation period (Weeks 1, 2, 3 and 4). Furthermore, as pregnancy continues, there is an obvious increase in the number of small follicles (< 1.7 mm) present in the ovaries. Each additional week of pregnancy reveals an increase in the number of small follicles in comparison to the non-pregnant animals. By week 3 and week 4 of pregnancy, there are almost 3 times as many small follicles (30 follicles/10 ovaries) as found in the intact, control animals (9 follicles/10 ovaries). All these data suggested that during pregnancy, there is a dissappearance of large follicles (> 1.7 mm) with a concomitant increase in the number of small follicles (< 1.7 mm).

Figure 1. Relationship Between the Number of Follicles of Different Sizes Recovered at Various Stages of Pregnancy.

On the ordinate are the external follicular diameters in millimeters (mm). On the abscissa are the weekly time periods during pregnancy, when follicles collection was made. Follicles less than 1.0 mm were not collected.

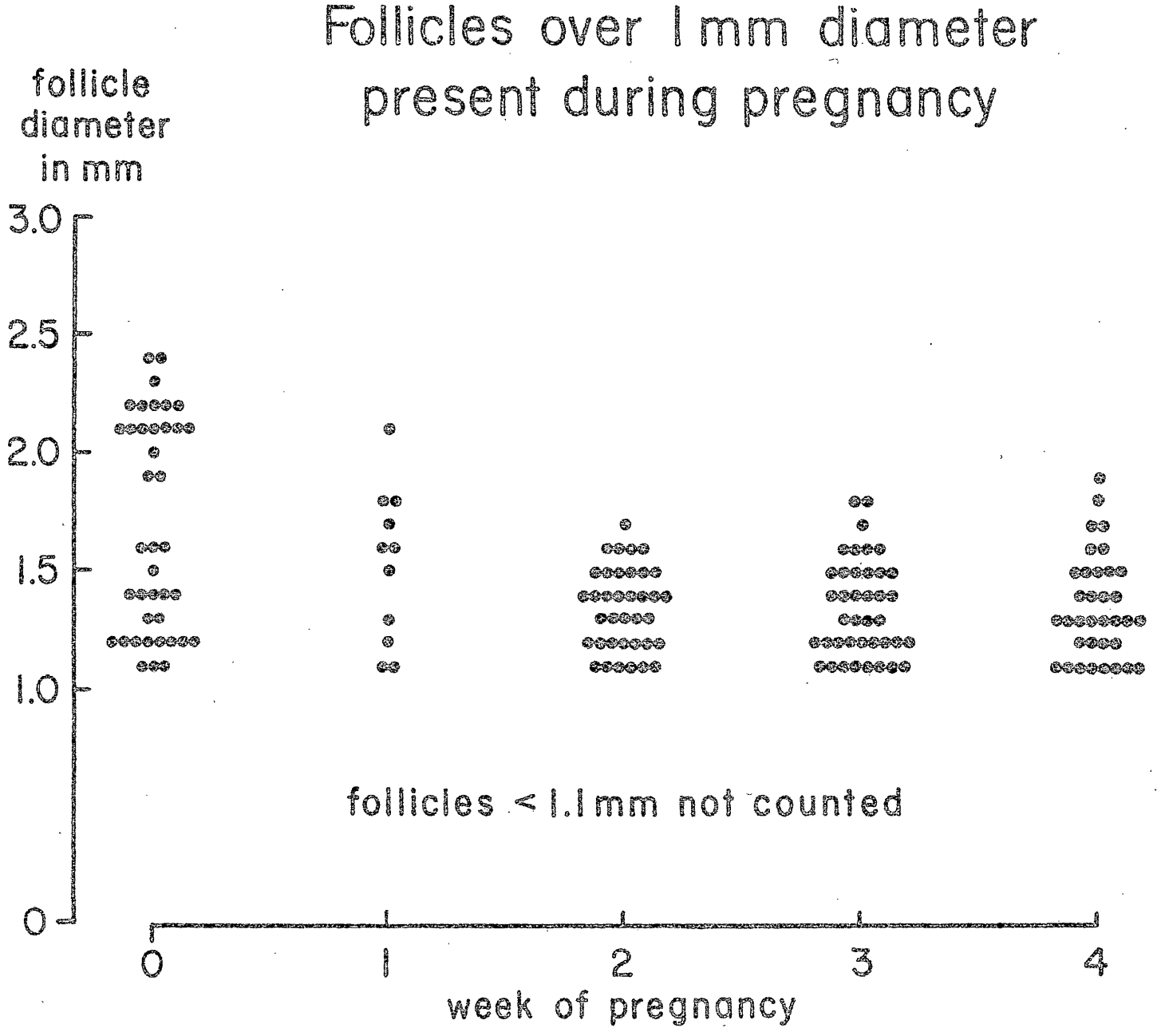
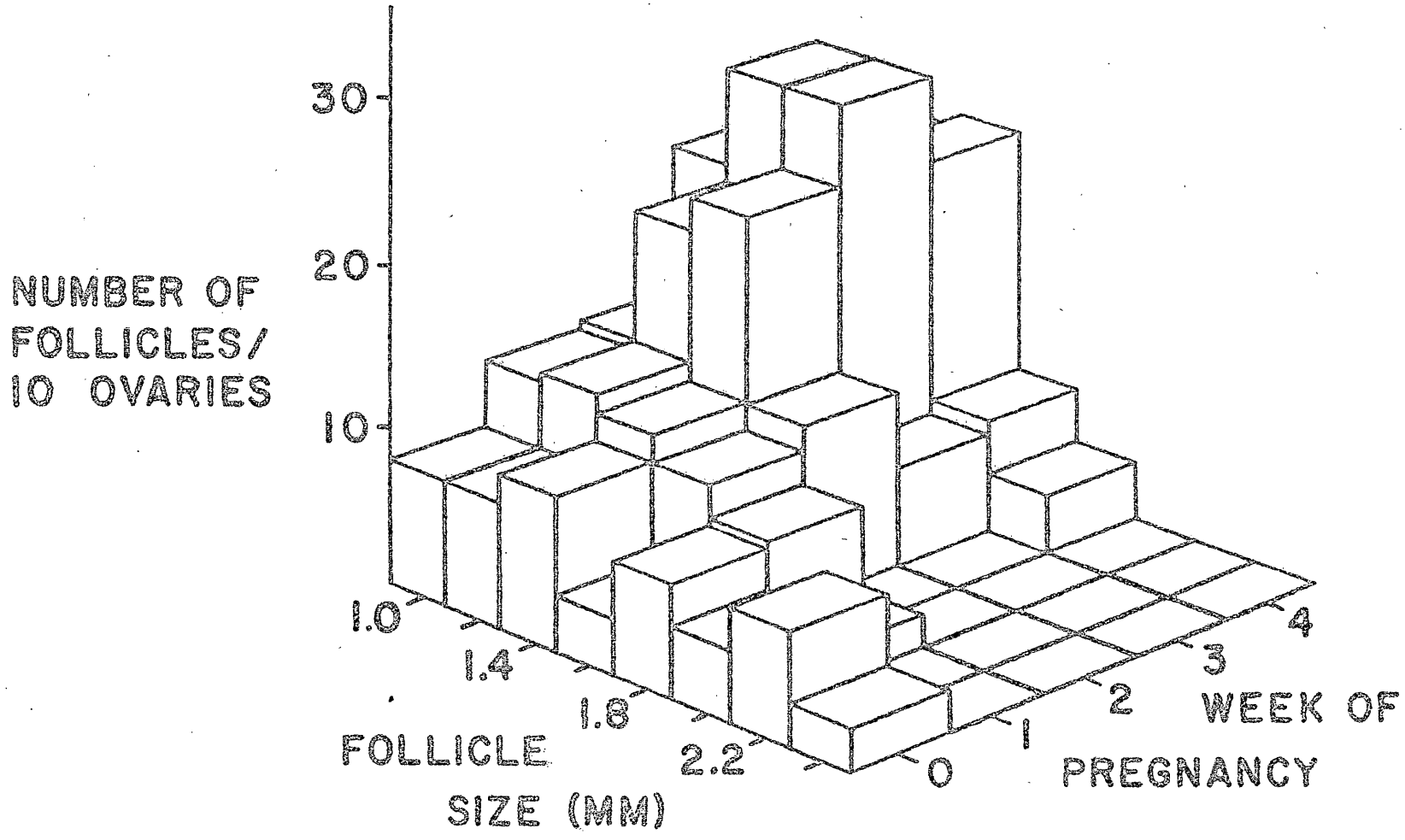

Follicles over 1 mm diameter present during pregnancy
follicle diameter in mm
3.0
2.5
2.0
1.5
1.0
0
follicles < 1.1 mm not counted
0
1
2
3
4
week of pregnancy

Figure 2. The Frequency Distribution of Follicles Collected at Weekly Intervals During Pregnancy.

On the X-axis is the follicular diameter in millimeters (mm). The Y-axis is the number of follicles collected per ten ovaries. The Z-axis represents the various stages of pregnancy. Note the absence of large follicles (> 1.7 mm) from week 1 through week 4 of pregnancy.


NUMBER OF FOLLICLES/ 10 OVARIES
30
20
10
1.0
1.4
1.8
2.2
FOLLICLE SIZE (MM)
0
1
2
3
4
WEEK OF PREGNANCY

Steroid Contents of Ovarian Follicles During Pregnancy

The content of estradiol and testosterone of the follicles collected at 0, 1, 2, 3, 4 weeks of pregnancy are presented in Figures 3-12. Among the estrous rabbits (0 week) both large follicles (> 1.7 mm) and small follicles (≤ 1.7 mm) were found to contain elevated quantities of both estradiol (Fig. 3) and testosterone (Fig. 4). However, in these nonpregnant animals the steroid content of the follicles do not seem to necessarily correlate with follicular size. Some large follicles display elevated levels of estrogen and testosterone while a few small follicles also exhibit elevated levels of individual steroid content.

At 1 week after mating, very few large follicles (> 1.7 mm) can be found. Even the large follicles that are present show relatively low contents of estradiol (Figure 5) and testosterone (Figure 6). The low steroid content is especially evident if compared to the estrogen and testosterone contents of non-pregnant animals (Figures 3 and 4). As pregnancy continues, with the obvious absence of large follicles (> 1.7 mm), the estradiol and testosterone content of follicles continues to be very low (Figures 7-12). There is little variability in content of either steroid throughout the duration of pregnancy.

Figure 3. The Total Estrogen Content of Individual Follicles Isolated from Non-Pregnant (Control) Rabbit Ovaries.

On the ordinate are total estrogen content per individual follicle in picograms. On the abscissa are the external diameters of the follicles in millimeters (mm). Follicles less than 1.0 mm in diameter were not collected.

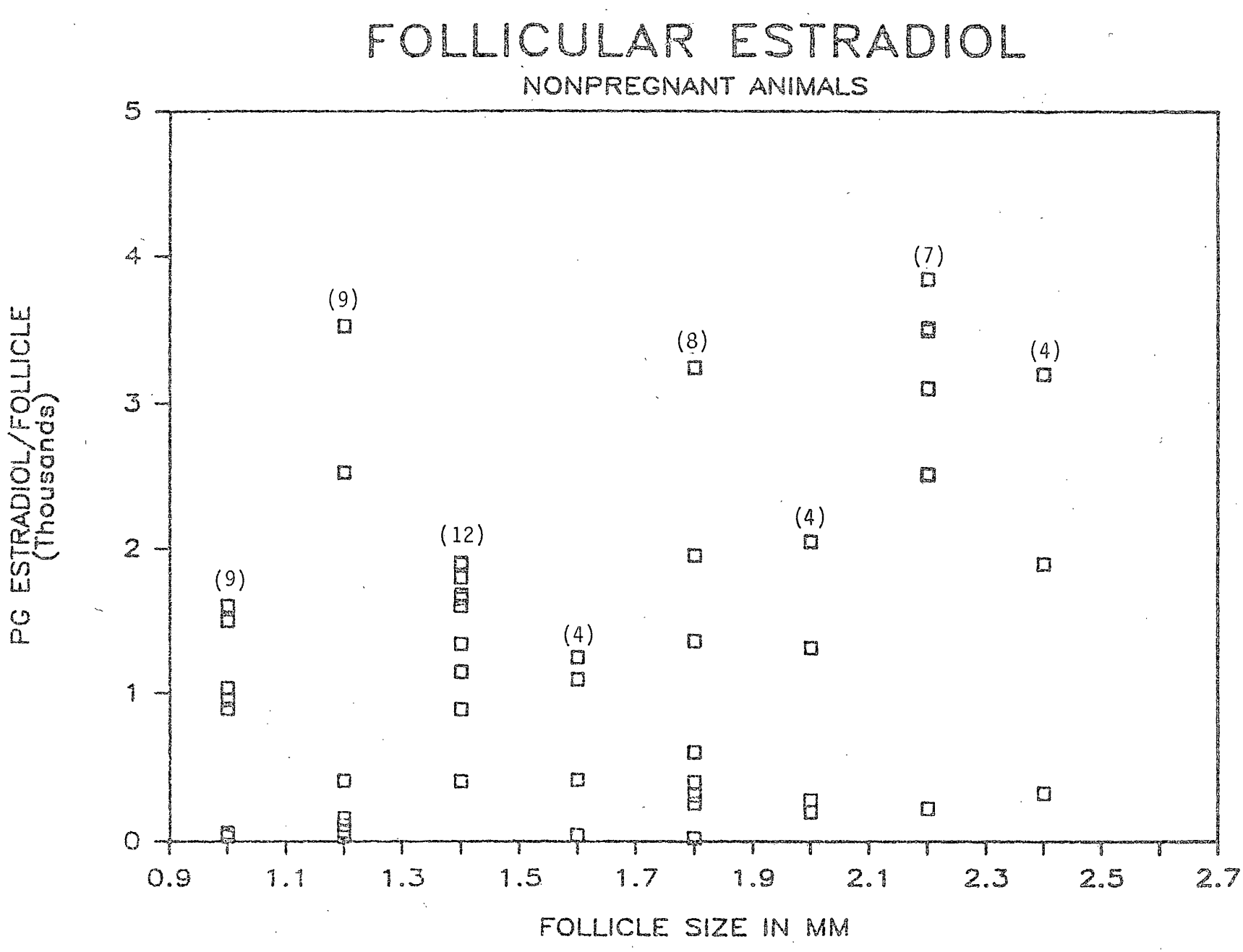

FOLLICULAR ESTRADIOL
NONPREGNANT ANIMALS
PG ESTRADIOL/FOLLICLE (Thousands)
FOLLICLE SIZE IN MM
0
1
2
3
4
5
0.9
1.1
1.3
1.5
1.7
1.9
2.1
2.3
2.5
2.7
(9)
(9)
(12)
(4)
(8)
(4)
(7)
(4)

Figure 4. The Total Testosterone Content of Individual Follicles Isolated from Non-Pregnant (Control) Rabbit Ovaries.

On the ordinate are total testosterone content per individual follicle in picograms. On the abscissa are the external diameters of the follicles in millimeters (mm). Follicles less than 1.0 mm in diameter were not collected.

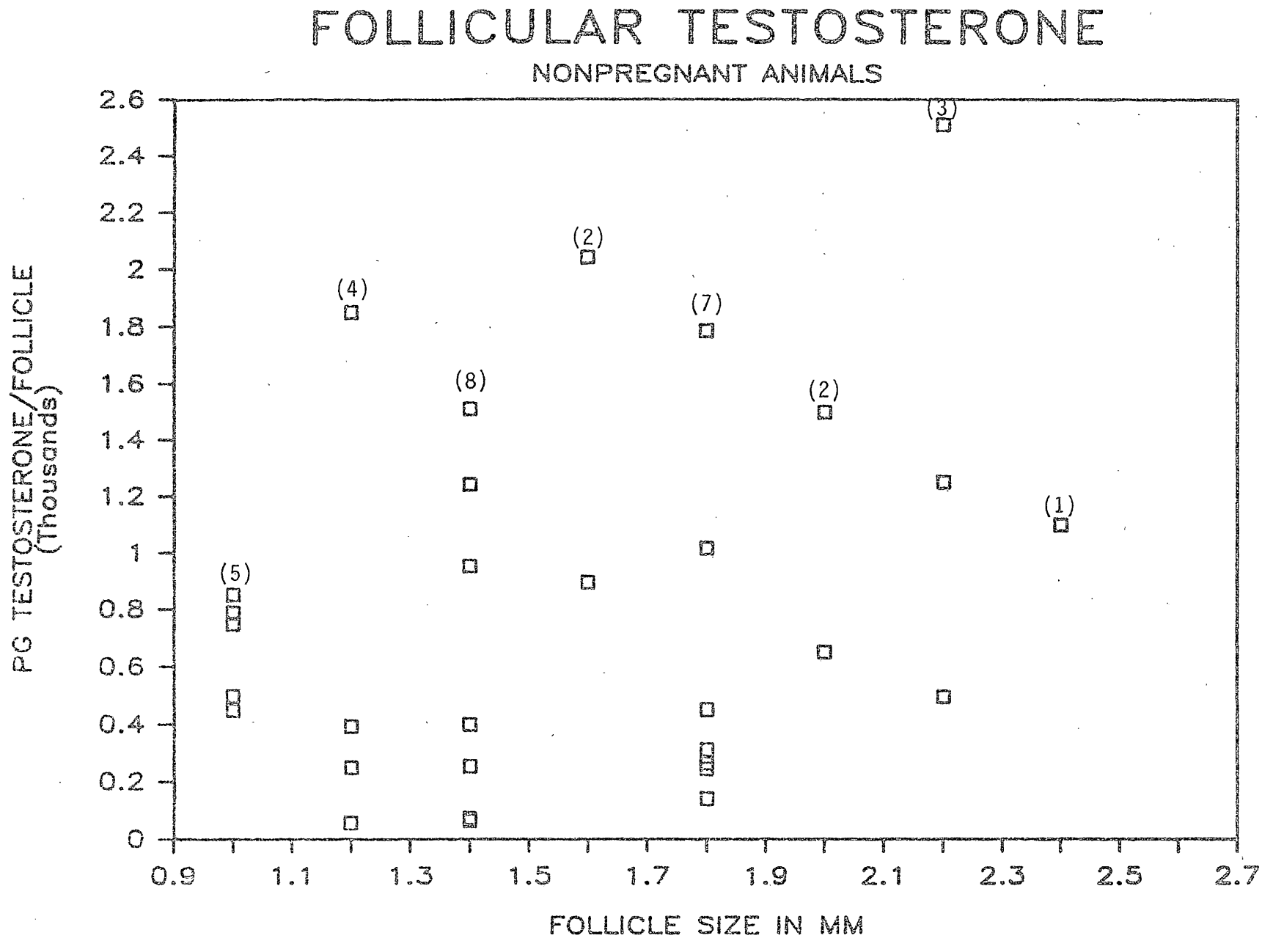

FOLLICULAR TESTOSTERONE
NONPREGNANT ANIMALS
PG TESTOSTERONE/FOLLICLE (Thousands)
2.6
2.4
2.2
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
(3)
(2)
(4)
(7)
(8)
(2)
(1)
(5)
0.9
1.1
1.3
1.5
1.7
1.9
2.1
2.3
2.5
2.7
FOLLICLE SIZE IN MM

Figure 5. The Total Estrogen Content of Individual Follicles Isolated from One Week Pregnant Rabbit Ovaries.

On the ordinate are total estrogen content per individual follicle in picograms. On the abscissa are the external diameters of the follicles in millimeters (mm). Follicles less than 1.0 mm in diameter were not collected.

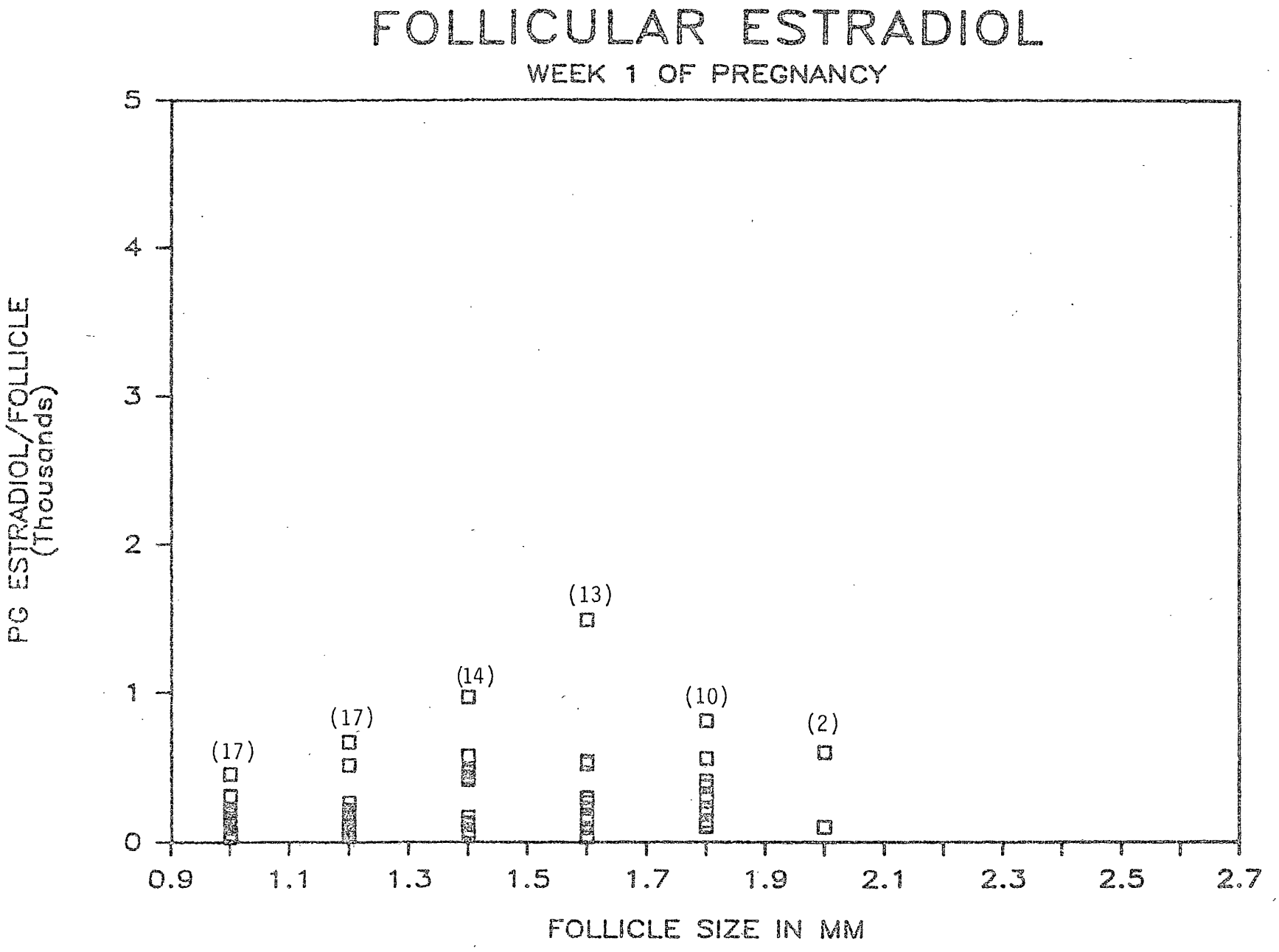

FOLLICULAR ESTRADIOL
WEEK 1 OF PREGNANCY
PG ESTRADIOL/FOLLICLE (Thousands)
0
1
2
3
4
5
(17)
(17)
(14)
(13)
(10)
(2)
0.9
1.1
1.3
1.5
1.7
1.9
2.1
2.3
2.5
2.7
FOLLICLE SIZE IN MM

Figure 6. The Total Testosterone Content of Individual Follicles Isolated from One Week Pregnant Rabbit Ovaries.

On the ordinate are total testosterone content per individual follicle in picograms. On the abscissa are the external diameters of the follicles in millimeters (mm). Follicles less than 1.0 mm in diameter were not collected.

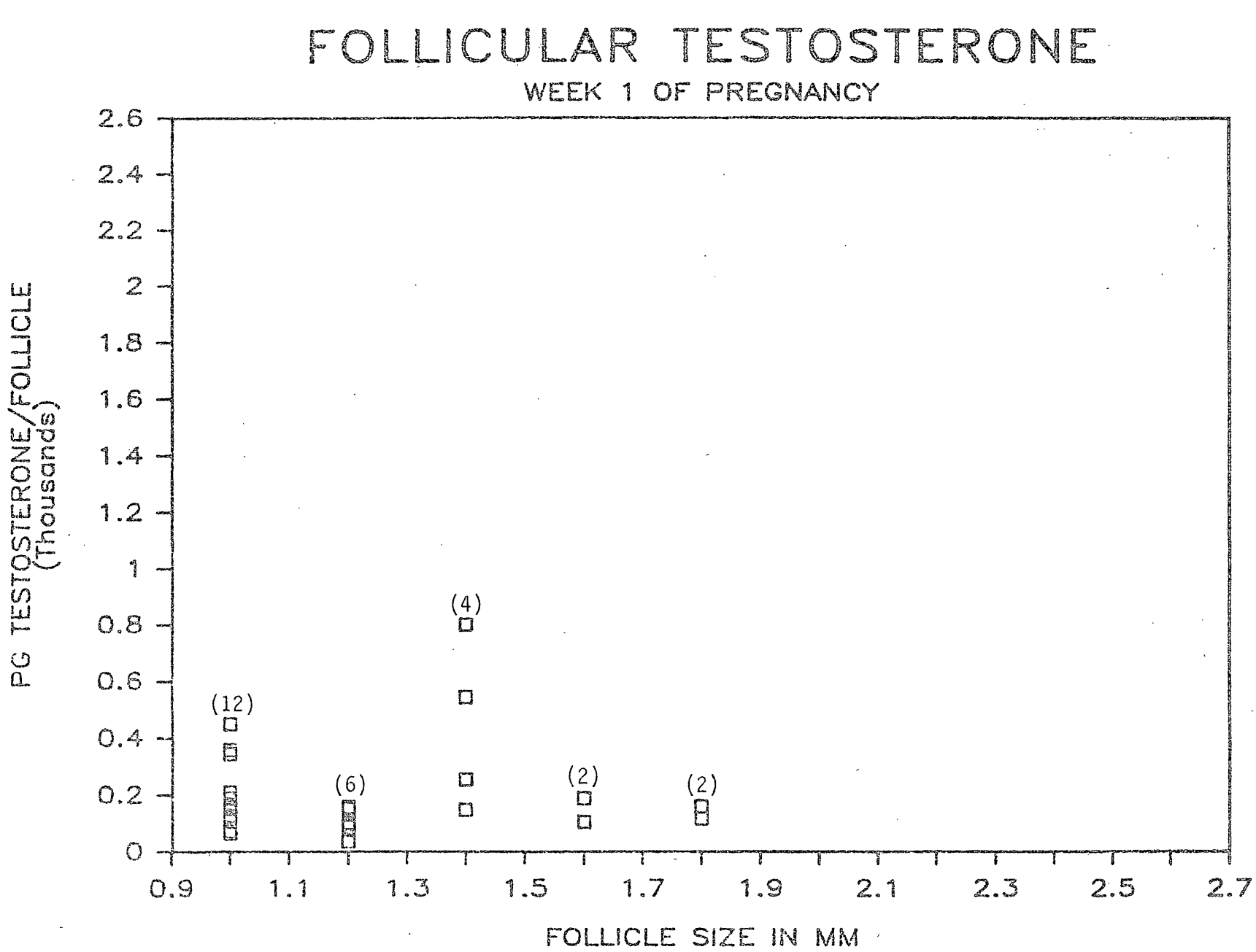

FOLLICULAR TESTOSTERONE
WEEK 1 OF PREGNANCY
PG TESTOSTERONE/FOLLICLE (Thousands)
2.6
2.4
2.2
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
(12)
(6)
(4)
(2)
(2)
0.9
1.1
1.3
1.5
1.7
1.9
2.1
2.3
2.5
2.7
FOLLICLE SIZE IN MM

Figure 7. The Total Estrogen Content of Individual Follicles Isolated from Two Week Pregnant Rabbit Ovaries.

On the ordinate are total estrogen content per individual follicle in picograms. On the abscissa are the external diameters of the follicles in millimeters (mm). Follicles less than 1.0 mm in diameter were not collected.

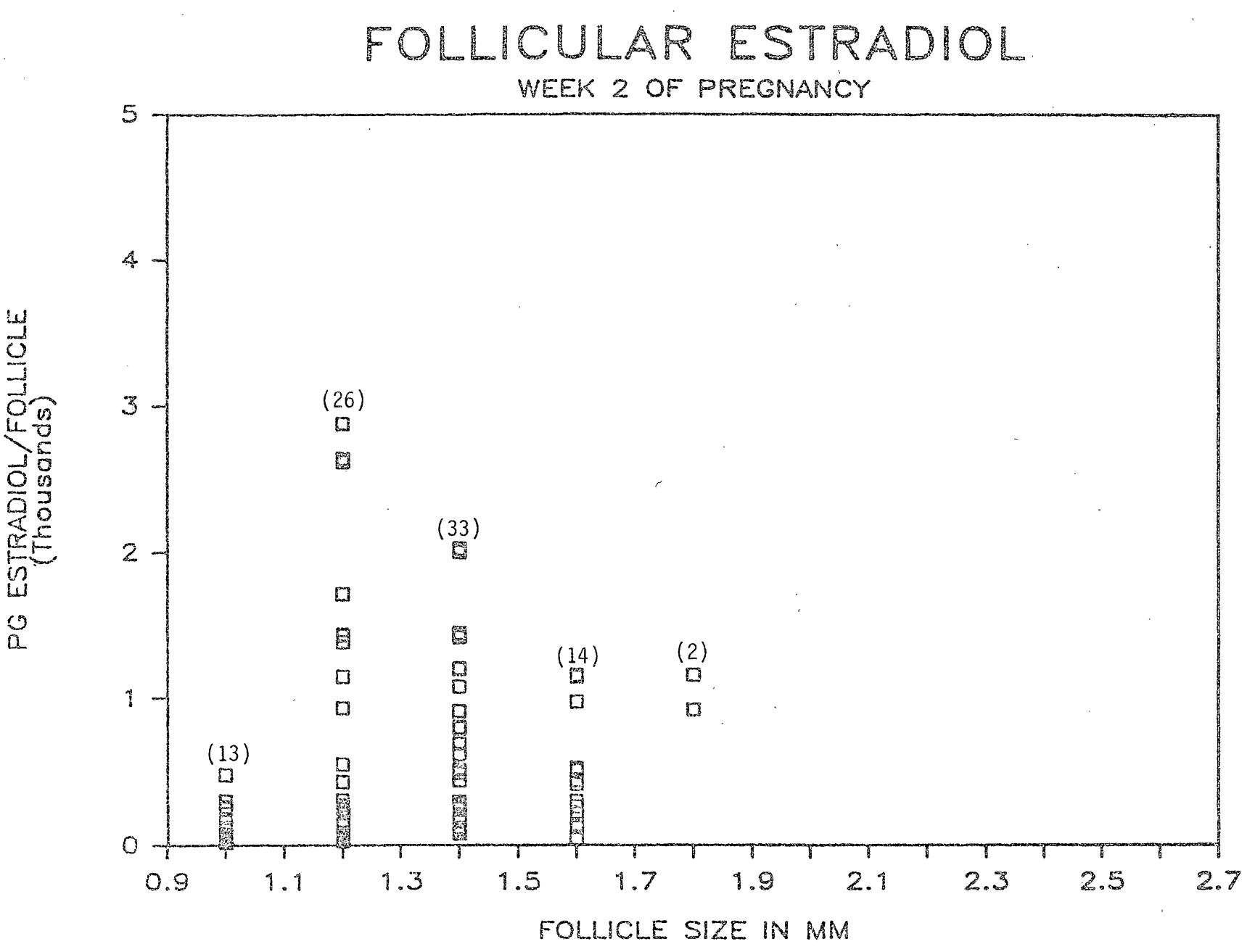

FOLLICULAR ESTRADIOL
WEEK 2 OF PREGNANCY
PG ESTRADIOL/FOLLICLE
(Thousands)
5
4
3
2
1
0
(13)
(26)
(33)
(14)
(2)
0.9
1.1
1.3
1.5
1.7
1.9
2.1
2.3
2.5
2.7
FOLLICLE SIZE IN MM

Figure 8. The Total Testosterone Content of Individual Follicles Isolated from Two Week Pregnant Rabbit Ovaries.

On the ordinate are total testosterone content per individual follicle in picograms. On the abscissa are the external diameters of the follicles in millimeters (mm). Follicles less than 1.0 mm in diameter were not collected.

FOLLICULAR TESTOSTERONE

WEEK 2 OF PREGNANCY

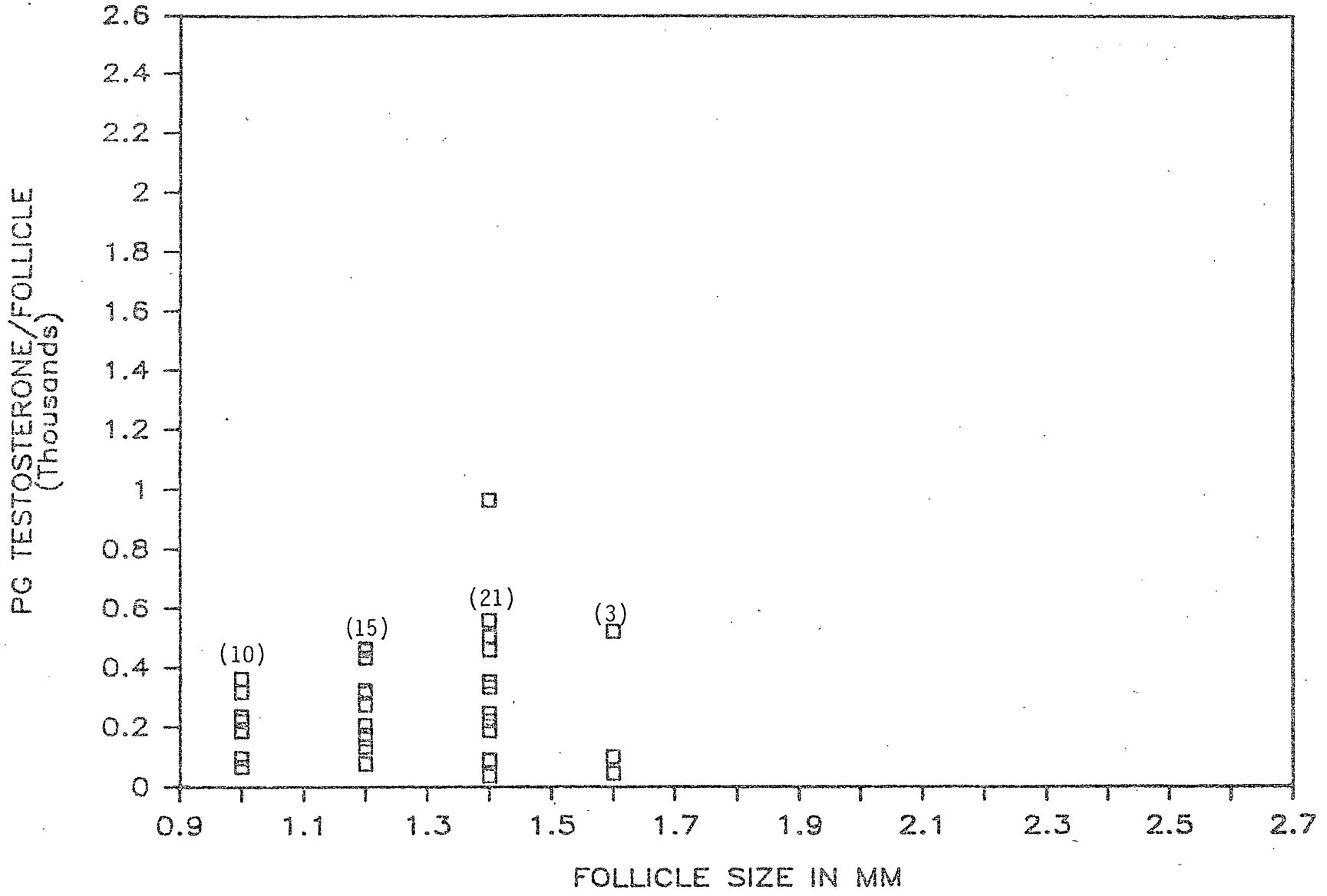


Figure 9. The Total Estrogen Content of Individual Follicles Isolated from Three Week Pregnant Rabbit Ovaries.

On the ordinate are total estrogen content per individual follicle in picograms. On the abscissa are the external diameters of the follicles in millimeters (mm). Follicles less than 1.0 mm in diameter were not collected.

FOLLICULAR ESTRADIOL

WEEK 3 OF PREGNANCY

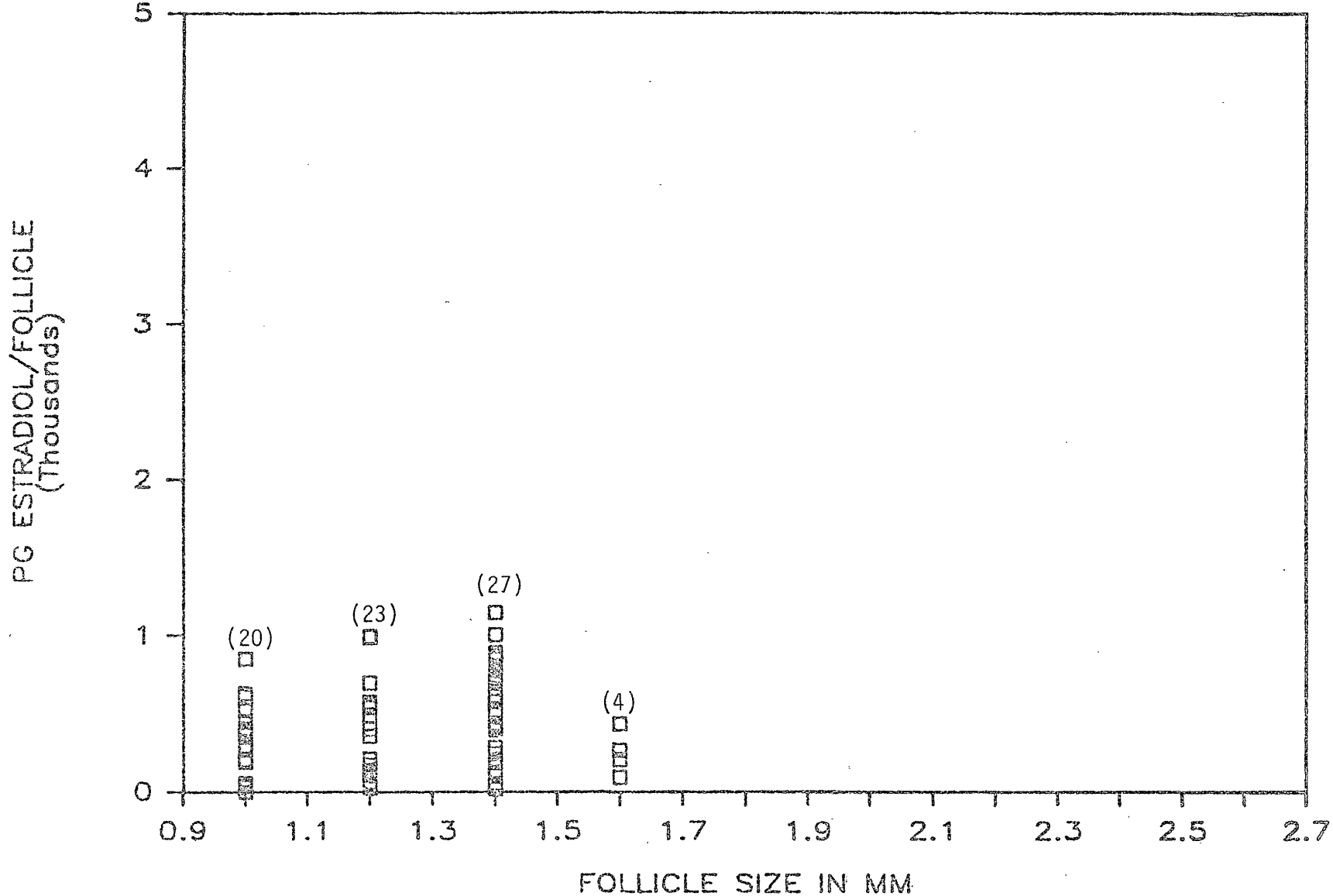


Figure 10. The Total Testosterone Content of Individual Follicles Isolated from Three Week Pregnant Rabbit Ovaries.

On the ordinate are total testosterone content per individual follicle in picograms. On the abscissa are the external diameters of the follicles in millimeters (mm). Follicles less than 1.0 mm in diameter were not collected.

FOLLICULAR TESTOSTERONE

WEEK 3 OF PREGNANCY

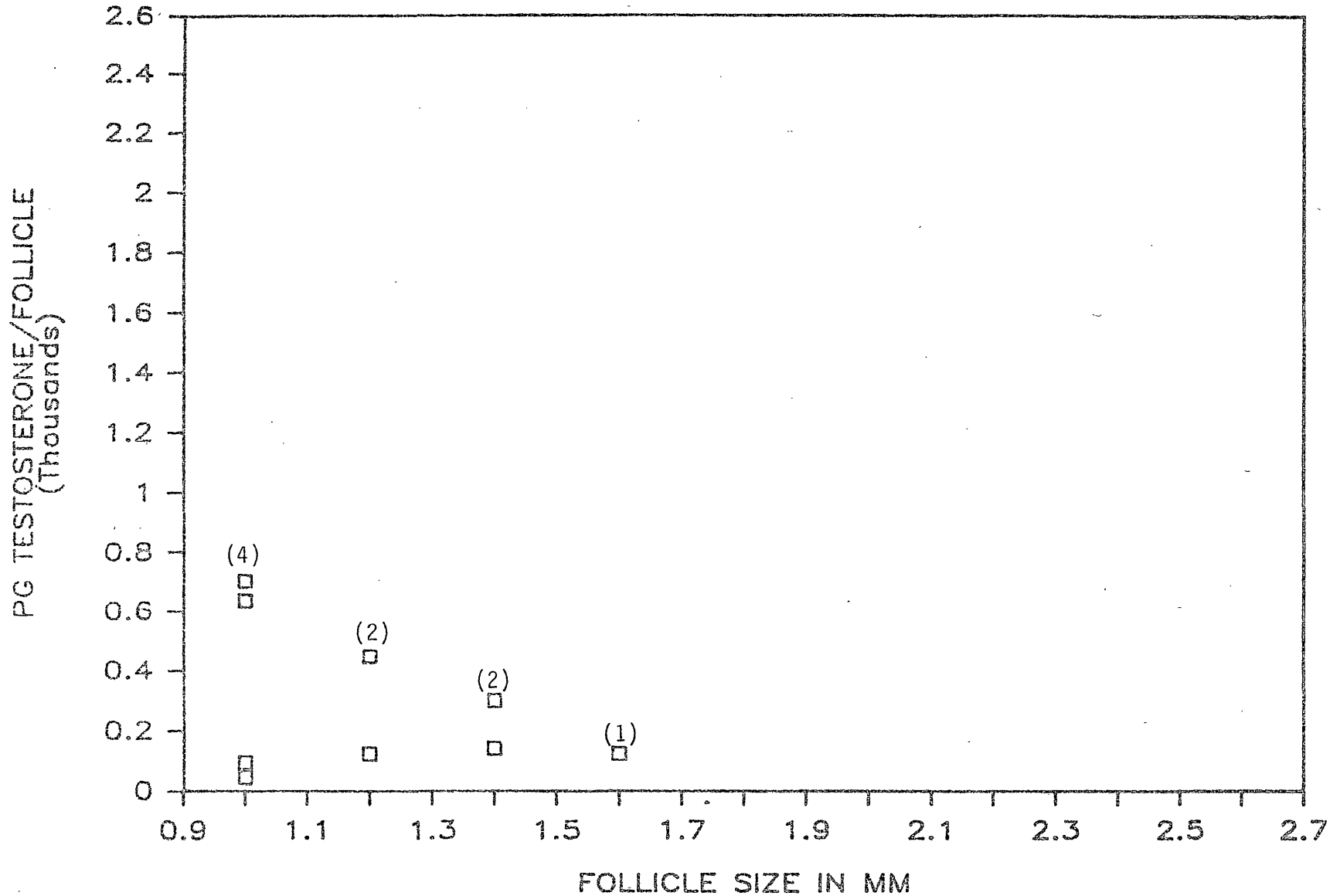


Figure 11. The Total Estrogen Content of Individual Follicles Isolated from Four Week Pregnant Rabbit Ovaries.

On the ordinate are total estrogen content per individual follicle in picograms. On the abscissa are the external diameters of the follicles in millimeters (mm). Follicles less than 1.0 mm in diameter were not collected.

FOLLICULAR ESTRADIOL

WEEK 4 OF PREGNANCY

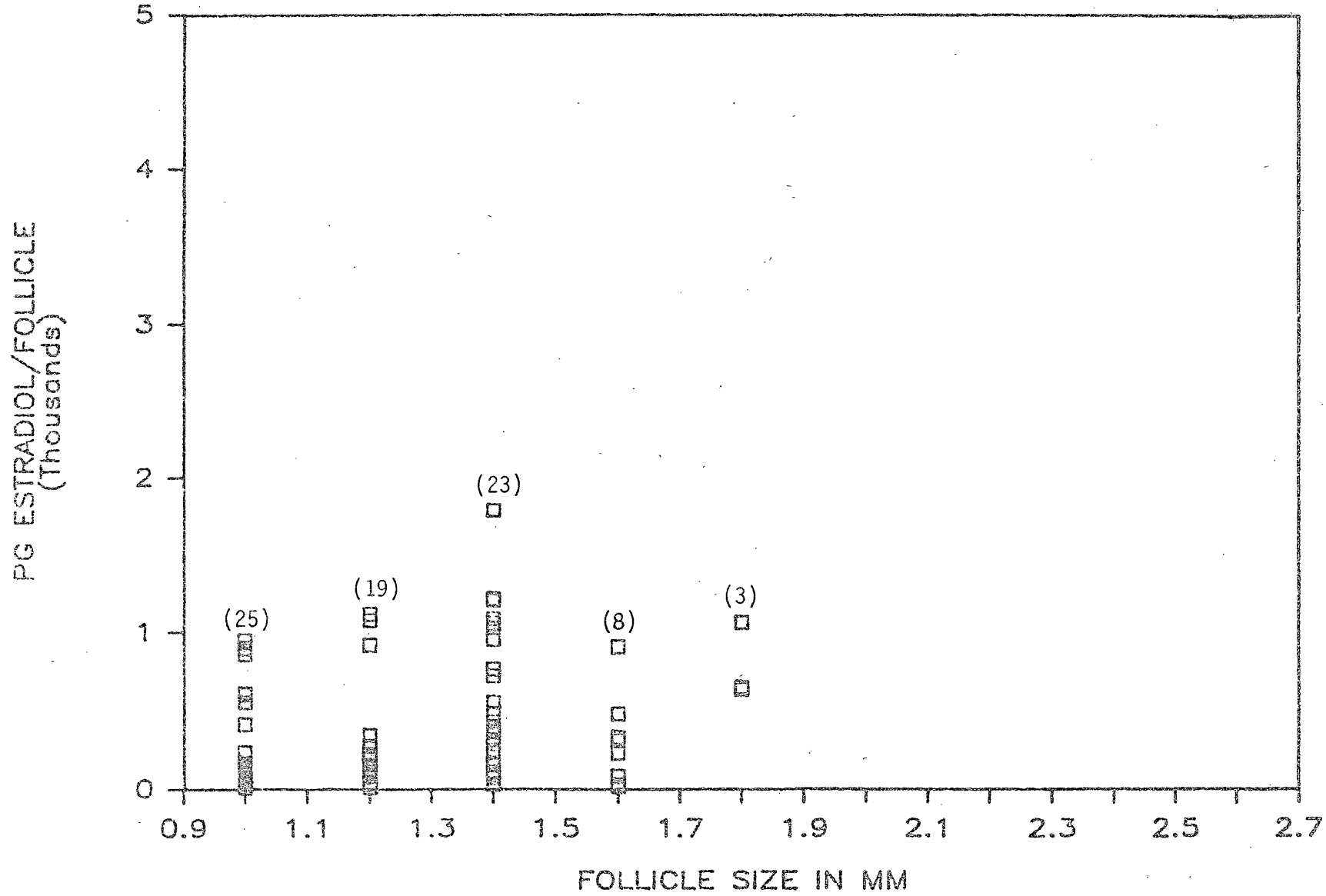
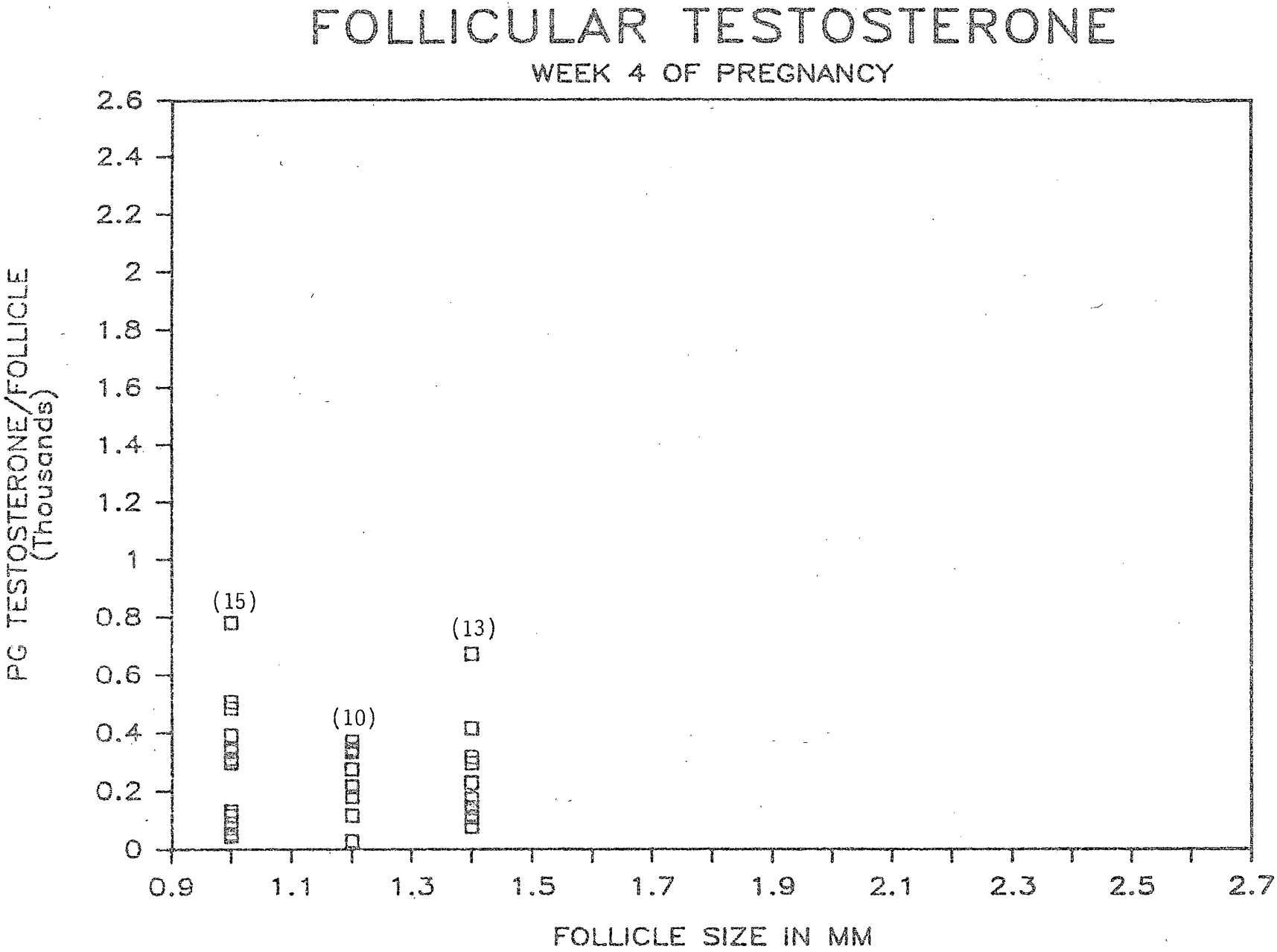


Figure 12. The Total Testosterone Content of Individual Follicles Isolated from Four Week Pregnant Rabbit Ovaries.

On the ordinate are total testosterone content per individual follicle in picograms. On the abscissa are the external diameters of the follicles in millimeters (mm). Follicles less than 1.0 mm in diameter were not collected.


FOLLICULAR TESTOSTERONE
WEEK 4 OF PREGNANCY
PG TESTOSTERONE/FOLLICLE
(Thousands)
2.6
2.4
2.2
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
(15)
(10)
(13)
0.9
1.1
1.3
1.5
1.7
1.9
2.1
2.3
2.5
2.7
FOLLICLE SIZE IN MM

Blood Levels of Progesterone, LH and FSH in Nonpregnant and Pregnant Rabbits

If high circulating levels of progesterone are a major regulatory factor in follicle growth, as has been postulated, then the site of action of the steroid becomes important. The progesterone could act on the hypothalamohypophyseal axis or directly on the ovary. If the steroid was exerting it's inhibitary influence on the hypothalamus or pituitary, then elevated progesterone would be expected to be concomitantly suppressing circulating levels of LH and FSH.

Blood samples were collected for hormone analyses immediately before mating (day 0) and then again on day 14 of pregnancy in the same animals. By using the same animals and collecting blood samples, before mating and while pregnant, each animal could serve as it's own control and the resulting serum concentrations of hormones could be compared employing paired variable statistics.

Results obtained from this study are presented in Table 1. Progesterone was significantly elevated on the fourteenth day of pregnancy (11.3 ng/ml $\pm$ 1.2 ng/ml) as compared to the same animals before mating (0.4 ng/ml $\pm$ 0.1 ng/ml). Suprisingly, blood FSH levels, rather than displaying a suppression, were significantly increased in pregnant animals (4.3 $\pm$ 0.2 ng/ml) when compared to non-pregnant animals (3.5 $\pm$ 0.1 ng/ml). LH levels were not different before or after mating (Table 1). Neither was there a significant difference in levels of blood estradiol.

Table 1. The Levels of Progesterone, FSH and LH in Blood Samples Collected from the Same Rabbits Before Mating and on Day 14 of Pregnancy.

Hormone	Before Mating	Day 14 of Pregnancy
	ng/ml serum	
progesterone	0.4 ± 0.1	11.3 ± 1.2*
FSH	3.5 ± 0.1	4.3 ± 0.2*
LH	0.5 ± 0.1	0.8 ± 0.3

*Significantly greater than before mating ($P < 0.05$)

Progesterone Pellet Implantation

Serum levels of progesterone were measured in normal, intact does that had undergone progesterone pellet implantation. Each pellet contained 50 mg of compressed crystalline progesterone. In order to determine the exact number of pellets needed to elevate serum progesterone levels to those comparable with pregnant rabbits, 0, 1, 2, 4 or 8 pellets were placed in individual non-pregnant rabbits and serial blood collections were made. Results from the progesterone assays on these samples are presented in Table 2. There was a positive correlation between the number of progesterone pellets implanted and blood levels of progesterone. Eight pellets placed in two separate points in the nape of intact, non-pregnant rabbits, elevated serum progesterone levels to approximately 5-8 ng/ml (Table 2).

Table 2. Serum Levels of Progesterone After Progesterone Pellet Implantation.

Number of Pellets Used	Progesterone ng/ml serum
1	0.34 ng/ml
2	0.52 ng/ml
4	1.85 ng/ml
8	8.50 ng/ml

Characterization of the Size Distribution of Ovarian Follicles During Progesterone Treatment

Eight progesterone pellets were implanted in intact, non-pregnant animals, in order to elevate circulating levels of progesterone to levels similar to pregnancy concentrations. Does were sacrificed at week 0 (control) and weeks 1, 2, 3 and 4 of progesterone treatment. All follicles $\geq$ 1.0 mm were isolated, external diameters measured at each of these time intervals and then assayed individually for estradiol and testosterone content.

The results obtained are illustrated in Figure 13. In week 0 (control) animals, the ovaries contained follicles ranging in size from 1.0 mm to 2.5 mm. However, once progesterone treatment began, there was an absence of large follicles (> 1.7 mm) and this continued through progesterone treatment (weeks 1, 2, 3 and 4). While progesterone treatment prevented the development of large follicles, there appeared to be an increase in the number of small follicles (< 1.7 mm). Each consecutive week of progesterone treatment appeared to produce a gradual increase in the number of small follicles. It would seem that the pattern of follicle development in the progesterone-treated, nonpregnant rabbits is exactly the same as was seen in the pregnant rabbits. It can be concluded that progesterone inhibits follicular growth and development in the rabbit ovary.

Follicles obtained from these progesterone treated rabbits were assayed for estradiol and testosterone content (Figures 14-19). When compared to follicles from non-pregnant (0 week) animals (Figure 3), follicles from progesterone treated animals contained

smaller amounts of estradiol. This depressed secretion of estradiol through weeks 1-4 of progesterone treatment (Figures 14, 16, 18) was parallel to the estradiol secretion of follicles obtained from pregnant animals through weeks 1-4 (Figures 5, 7, 9, 11). Although a few follicles displayed elevated levels of estradiol, the majority of follicles from progesterone treated animals showed low levels of estradiol.

Testosterone content of individual follicles from animals treated with progesterone for 1, 2 and 4 weeks are displayed in Figures 15, 17, 19. As was evident in the estradiol content of these follicles, testosterone secretion was also suppressed by the progesterone treatment. Follicles from animals that received progesterone treatment (weeks 1-4) were determined to have concentrations of testosterone that are comparable to the levels of testosterone found in pregnant animals, weeks 1-4 (Figures 6, 8, 10 and 12).

Serum levels of progesterone were assayed in nonpregnant, pregnant and progesterone treated rabbits (Figure 20). Although progesterone levels were elevated in the pelleted animals (approximately 4.5 ng/ml), they were not elevated to the circulating levels of progesterone measured in pregnant animals (> 6 ng/ml). Blood levels of circulating gonadotropins in progesterone-treated rabbits (Figure 21) were identical to levels found in pregnant rabbits. Figure 23 summarizes all the results of follicles collected from ovaries of nonpregnant, pregnant, and progesterone-treated rabbits. As indicated in Figure 22, pregnant and progesterone treated rabbits exhibit a total lack of follicles

> 2.0 mm, which are very evident in nonpregnant animals.

It would seem that in terms of follicle development, animals treated with progesterone exhibited many of the same characteristics as pregnant animals. Progesterone treated animals, like their pregnant counterparts, showed a noticeable absence of large follicles (> 1.7 mm). There was also decreased concentrations of estradiol and testosterone in the progesterone treated animals, paralleling the follicular steroid concentrations found in pregnant animals. Since the follicles obtained from progesterone treated animals displayed several of the parameters found in follicles from pregnant animals, insertion of progesterone pellets had enabled us to construct an animal model which imitated pregnancy in terms of follicular growth.

Figure 13. The Frequency Distribution of Follicles Collected at Weekly Intervals During Progesterone Treatment.

On the X-axis is the follicular diameter in millimeters (mm). The Y-axis is the number of follicles collected per ten ovaries. The Z-axis represents the various weekly intervals of progesterone treatment. Note the absence of large follicles (> 1.7 mm) from week 1 through week 4 of progesterone treatment.

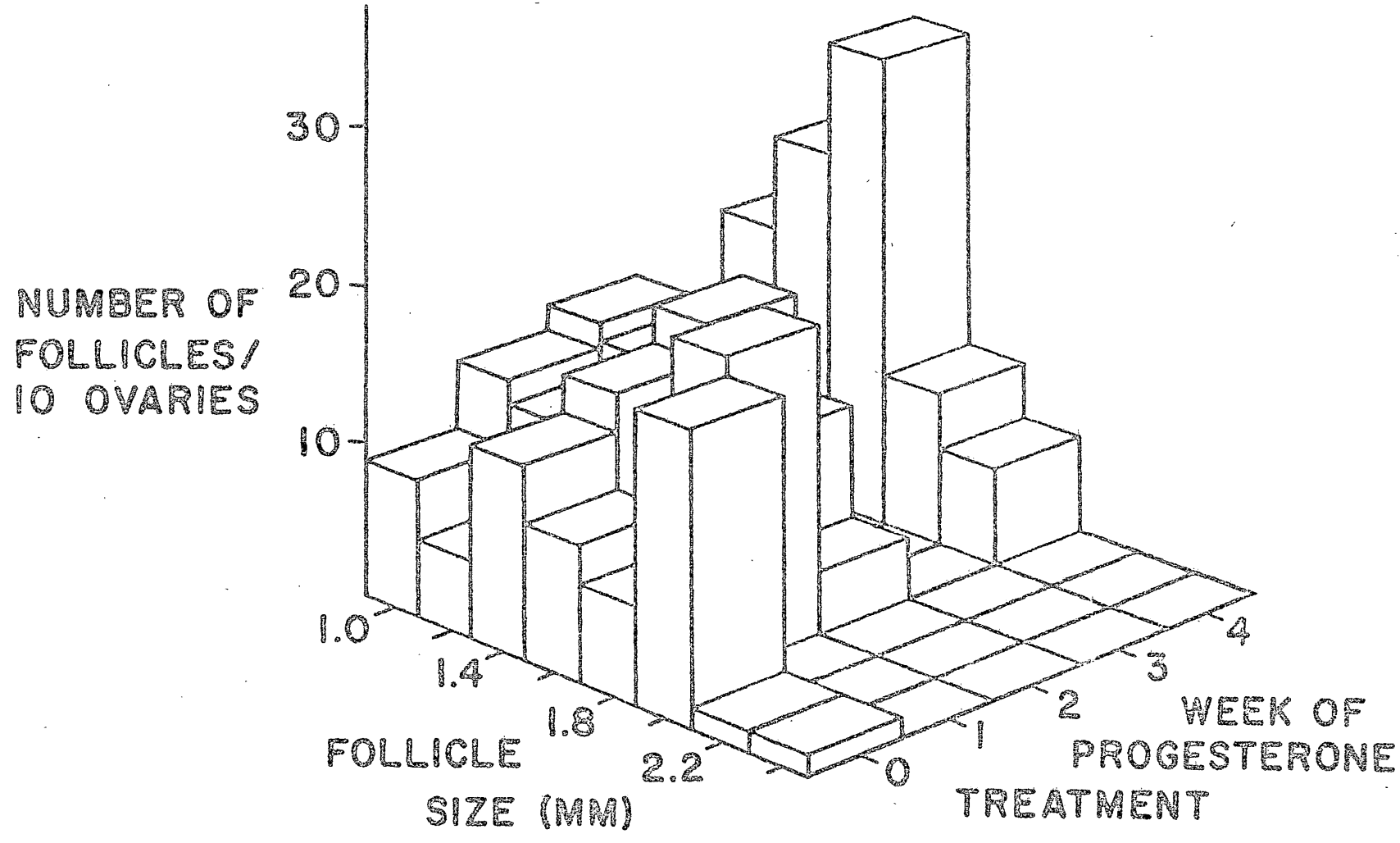

NUMBER OF FOLLICLES/ 10 OVARIES
30
20
10
1.0
1.4
1.8
2.2
FOLLICLE SIZE (MM)
0
1
2
3
4
WEEK OF PROGESTERONE TREATMENT

Figure 14. The Total Estrogen Content of Individual Follicles Isolated from One Week Progesterone Treated Rabbit Ovaries.

On the ordinate are total estrogen content per individual follicle in picograms. On the abscissa are the external diameters of the follicles in millimeters (mm). Follicles less than 1.0 mm in diameter were not collected.

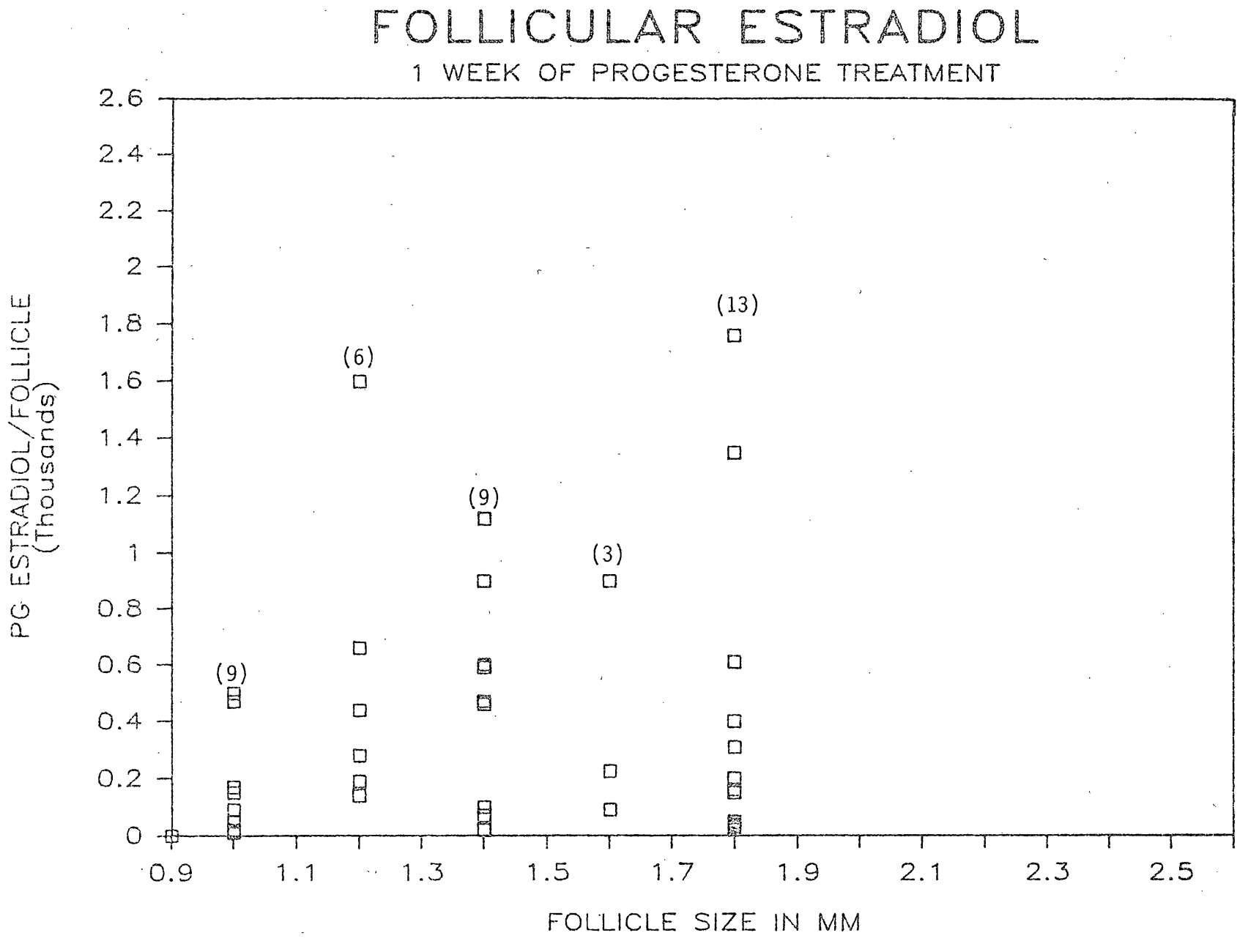

FOLLICULAR ESTRADIOL
1 WEEK OF PROGESTERONE TREATMENT
PG ESTRADIOL/FOLLICLE
(Thousands)
2.6
2.4
2.2
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
(9)
(6)
(9)
(3)
(13)
0.9
1.1
1.3
1.5
1.7
1.9
2.1
2.3
2.5
FOLLICLE SIZE IN MM

Figure 15. The Total Testosterone Content of Individual Follicles from One Week Progesterone Treated Rabbit Ovaries.

On the ordinate are total testosterone content per individual follicle in picograms. On the abscissa are the external diameters of the follicles in millimeters (mm). Follicles less than 1.0 mm in diameter were not collected.

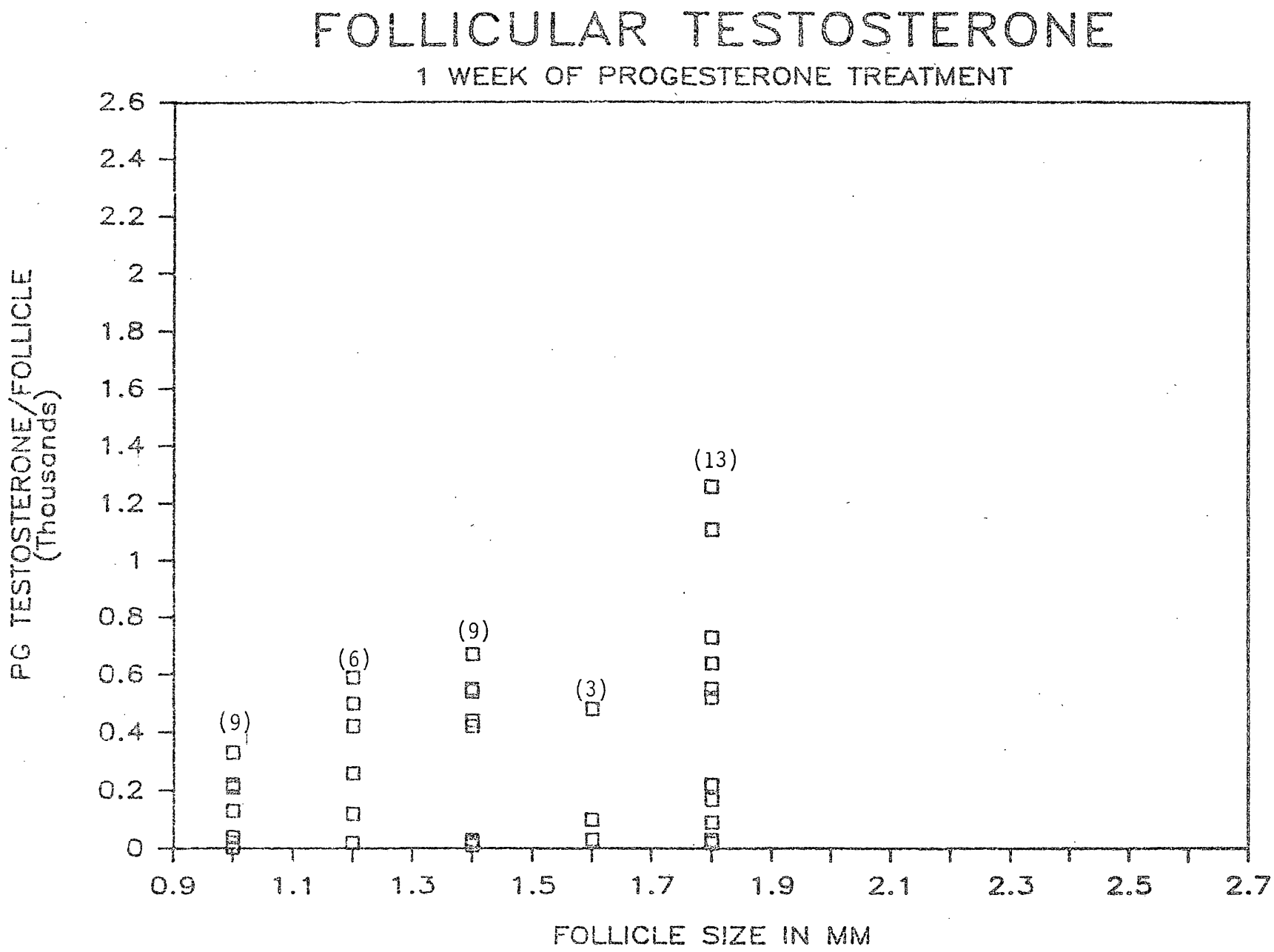

FOLLICULAR TESTOSTERONE
1 WEEK OF PROGESTERONE TREATMENT
PG TESTOSTERONE/FOLLICLE (Thousands)
2.6
2.4
2.2
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
(9)
(6)
(9)
(3)
(13)
0.9
1.1
1.3
1.5
1.7
1.9
2.1
2.3
2.5
2.7
FOLLICLE SIZE IN MM

Figure 16. The Total Estrogen Content of Individual Follicles Isolated from Two Week Progesterone Treated Rabbit Ovaries.

On the ordinate are total estrogen content per individual follicle in picograms. On the abscissa are the external diameters of the follicles in millimeters (mm). Follicles less than 1.0 mm in diameter were not collected.

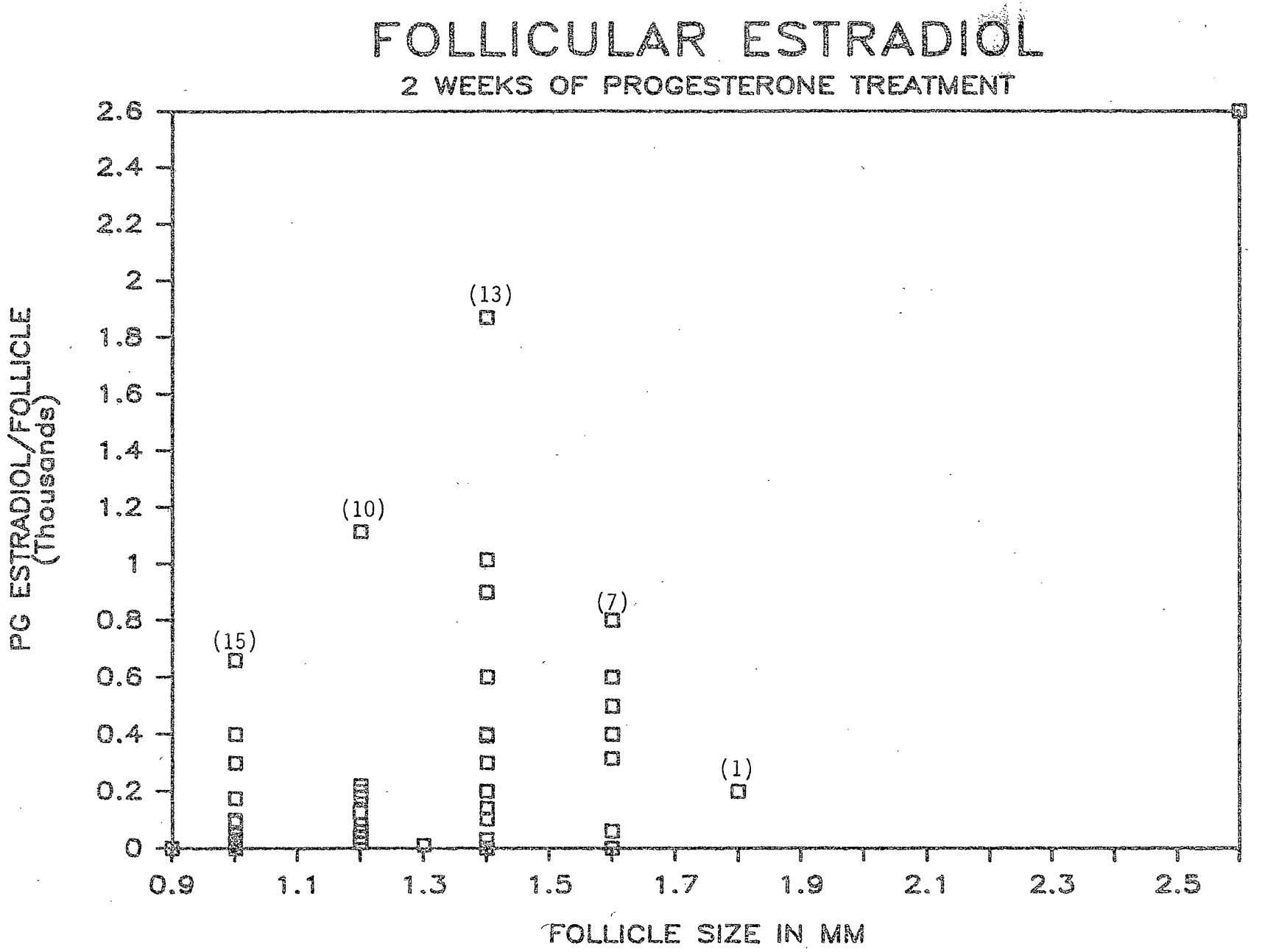

FOLLICULAR ESTRADIOL
2 WEEKS OF PROGESTERONE TREATMENT
PG ESTRADIOL/FOLLICLE (Thousands)
2.6
2.4
2.2
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
(13)
(10)
(7)
(15)
(1)
0.9
1.1
1.3
1.5
1.7
1.9
2.1
2.3
2.5
FOLLICLE SIZE IN MM

Figure 17. The Total Testosterone Content of Individual Follicles from Two Week Progesterone Treated Rabbit Ovaries.

On the ordinate are total testosterone content per individual follicle in picograms. On the abscissa are the external diameters of the follicles in millimeters (mm). Follicles less than 1.0 mm in diameter were not collected.

FOLLICULAR TESTOSTERONE

2 WEEKS OF PROGESTERONE TREATMENT

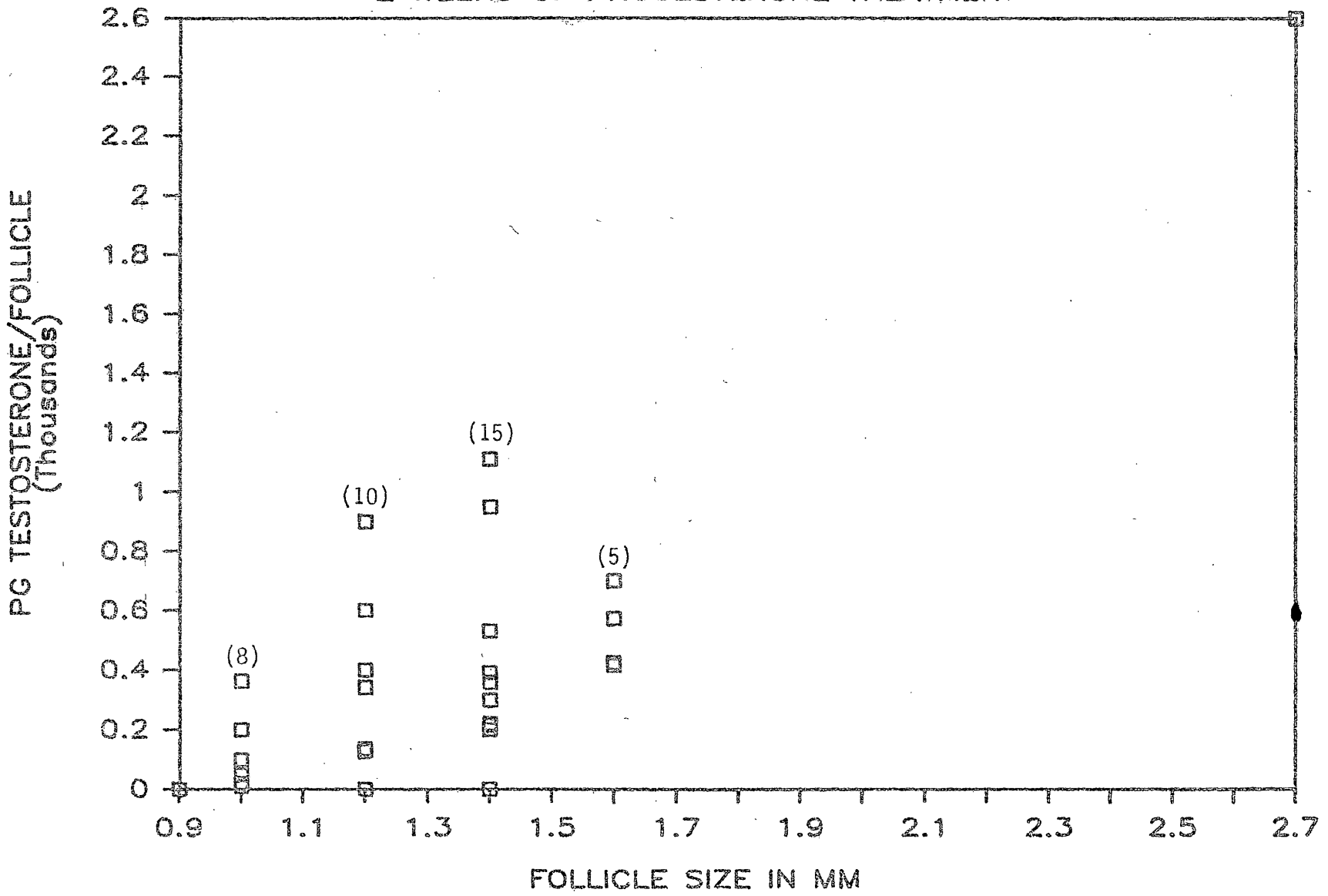


Figure 18. The Total Estrogen Content of Individual Follicles Isolated from Four Week Progesterone Treated Rabbit Ovaries.

On the ordinate are total estrogen content per individual follicle in picograms. On the abscissa are the external diameters of the follicles in millimeters (mm). Follicles less than 1.0 mm in diameter were not collected.

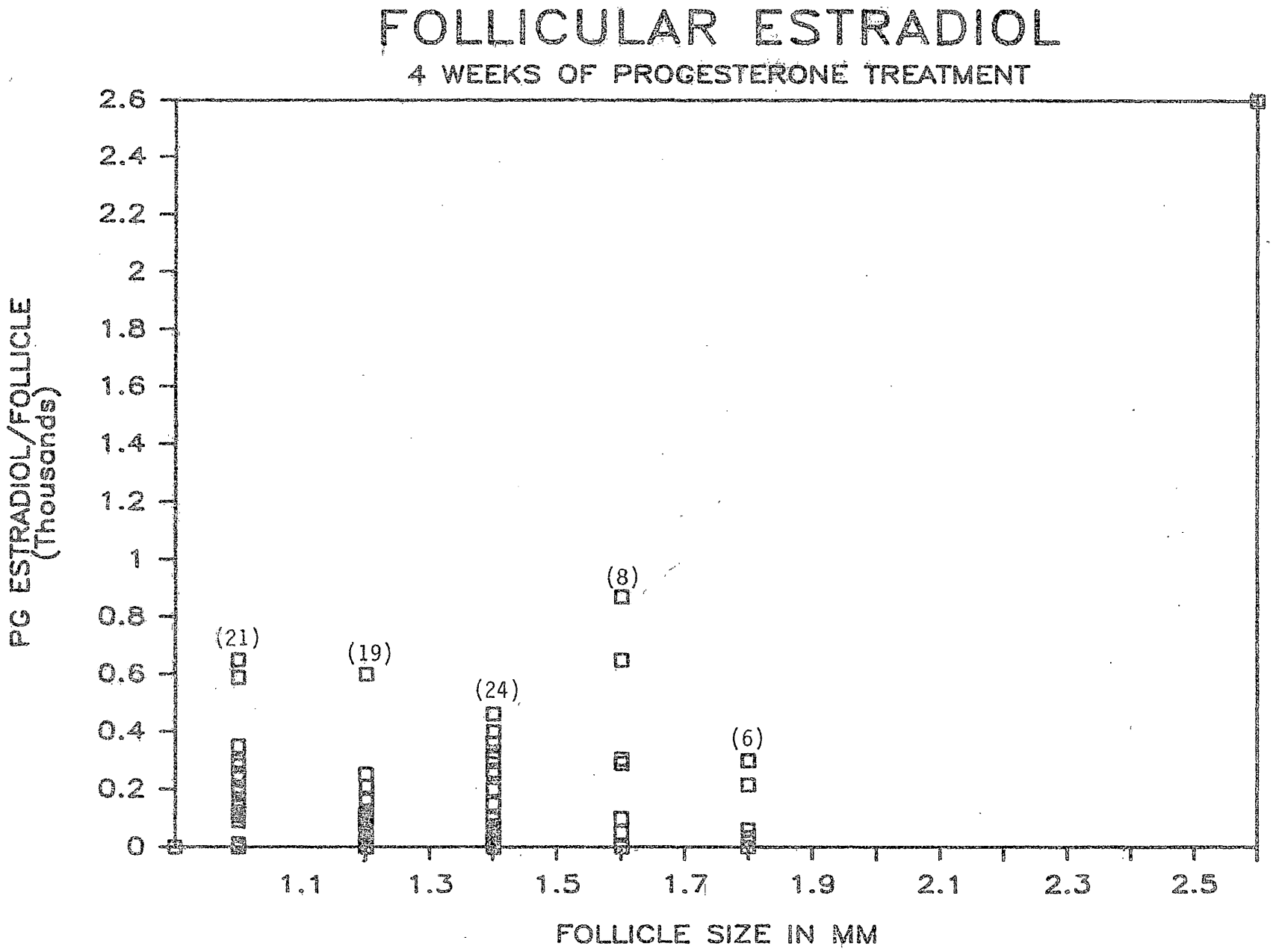

FOLLICULAR ESTRADIOL
4 WEEKS OF PROGESTERONE TREATMENT
PG ESTRADIOL/FOLLICLE
(Thousands)
2.6
2.4
2.2
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
(21)
(19)
(24)
(8)
(6)
1.1
1.3
1.5
1.7
1.9
2.1
2.3
2.5
FOLLICLE SIZE IN MM

Figure 19. The Total Testosterone Content of Individual Follicles from Four Week Progesterone Treated Rabbit Ovaries.

On the ordinate are total testosterone content per individual follicle in picograms. On the abscissa are the external diameters of the follicles in millimeters (mm). Follicles less than 1.0 mm in diameter were not collected.

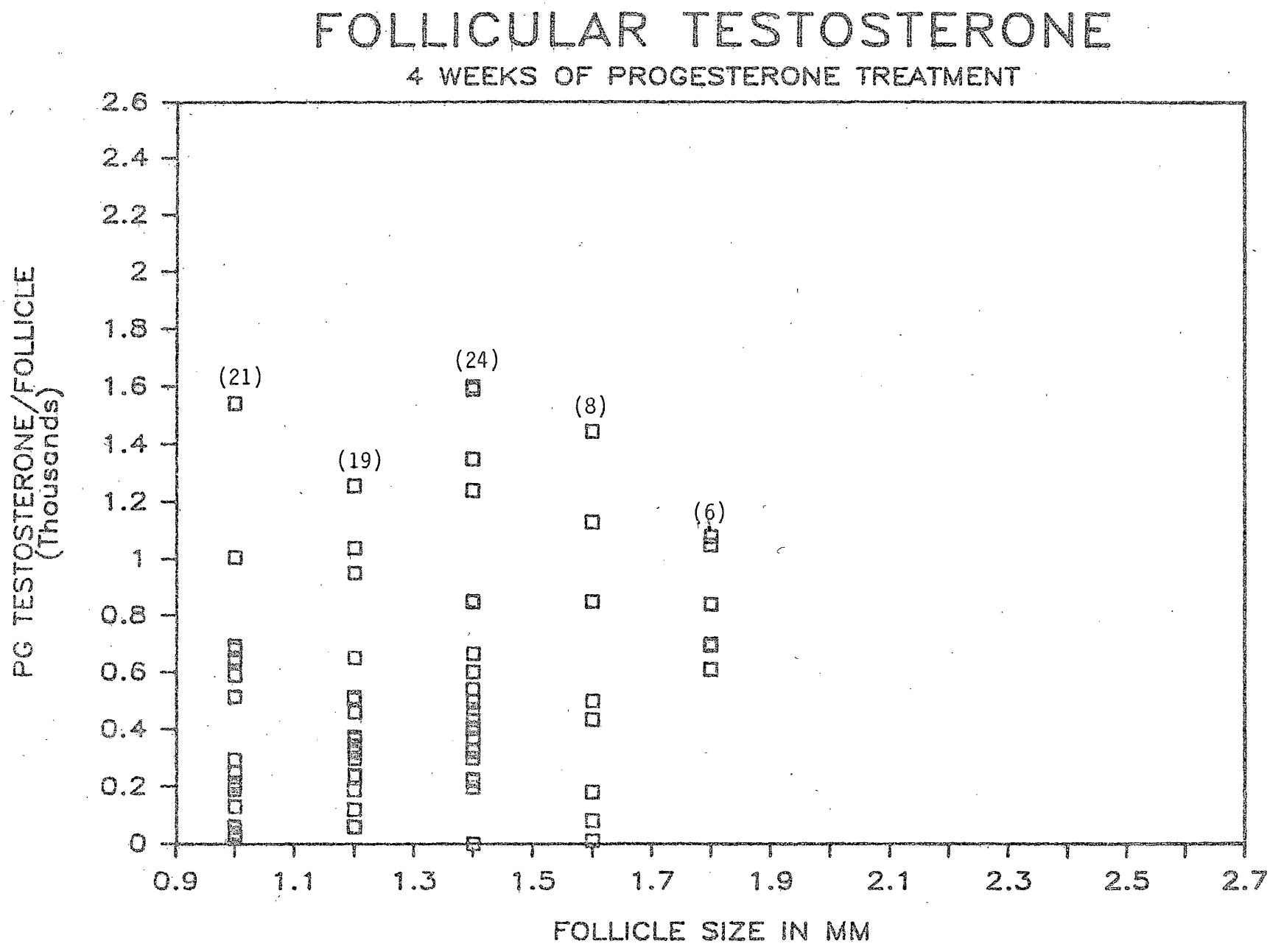

FOLLICULAR TESTOSTERONE
4 WEEKS OF PROGESTERONE TREATMENT
PG TESTOSTERONE/FOLLICLE (Thousands)
2.6
2.4
2.2
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
(21)
(19)
(24)
(8)
(6)
0.9
1.1
1.3
1.5
1.7
1.9
2.1
2.3
2.5
2.7
FOLLICLE SIZE IN MM

Figure 20. Serum Levels of Progesterone in Non-Pregnant (Control), Pregnant and Progesterone Treated Rabbits.

On the ordinate are levels of progesterone in nanograms per millileter of serum. On the abscissa are control (non-pregnant), pregnant and progesterone-pelleted rabbits.

BLOOD PROGESTERONE LEVELS

EFFECTS OF PREGNANCY AND P4 PELLETS

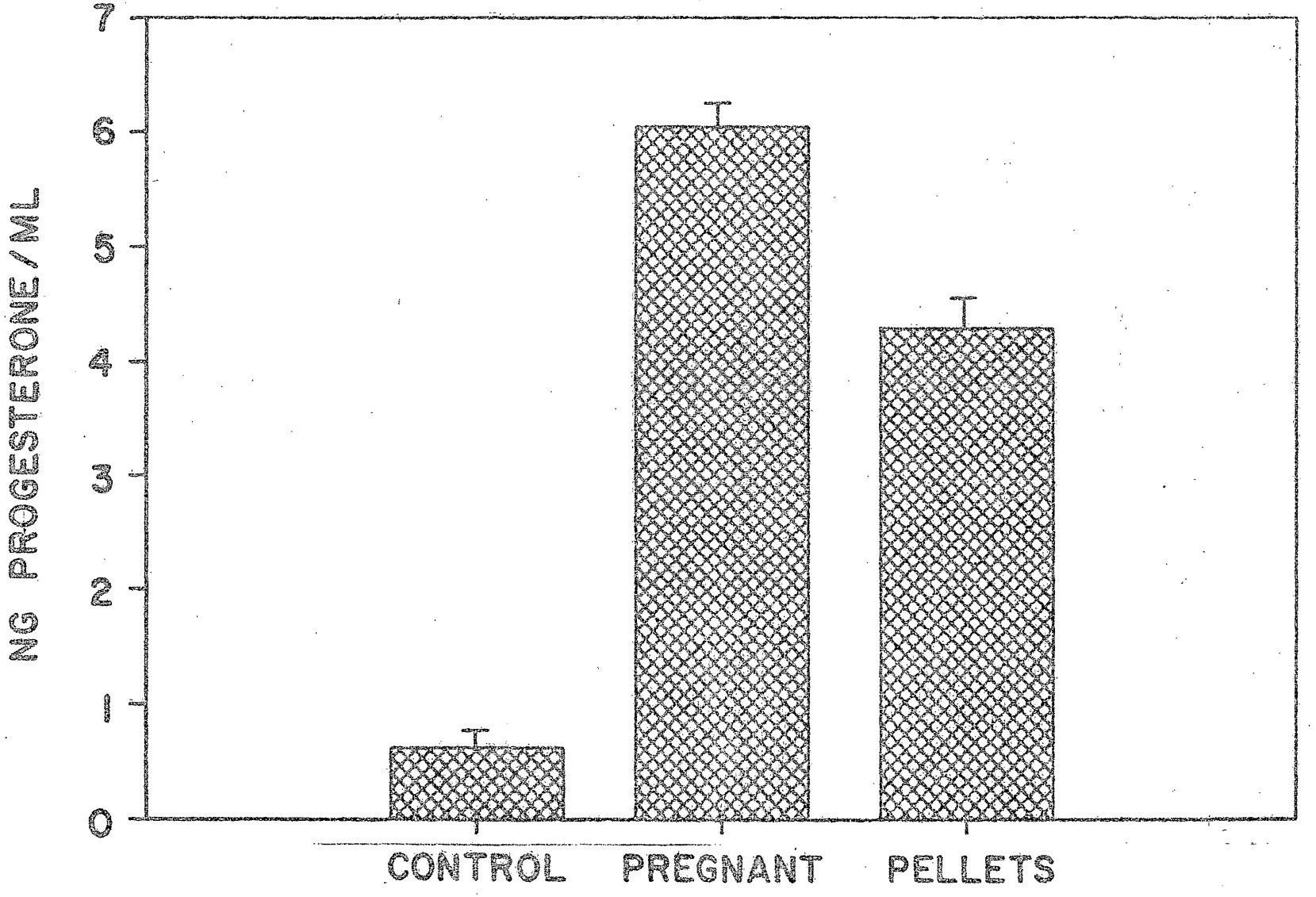


Figure 21. Serum Levels of FSH and LH in Progesterone Treated Rabbits.

On the ordinate are levels of FSH and LH in nanograms per millileter of serum. On the abscissa are days after pellet implantation.

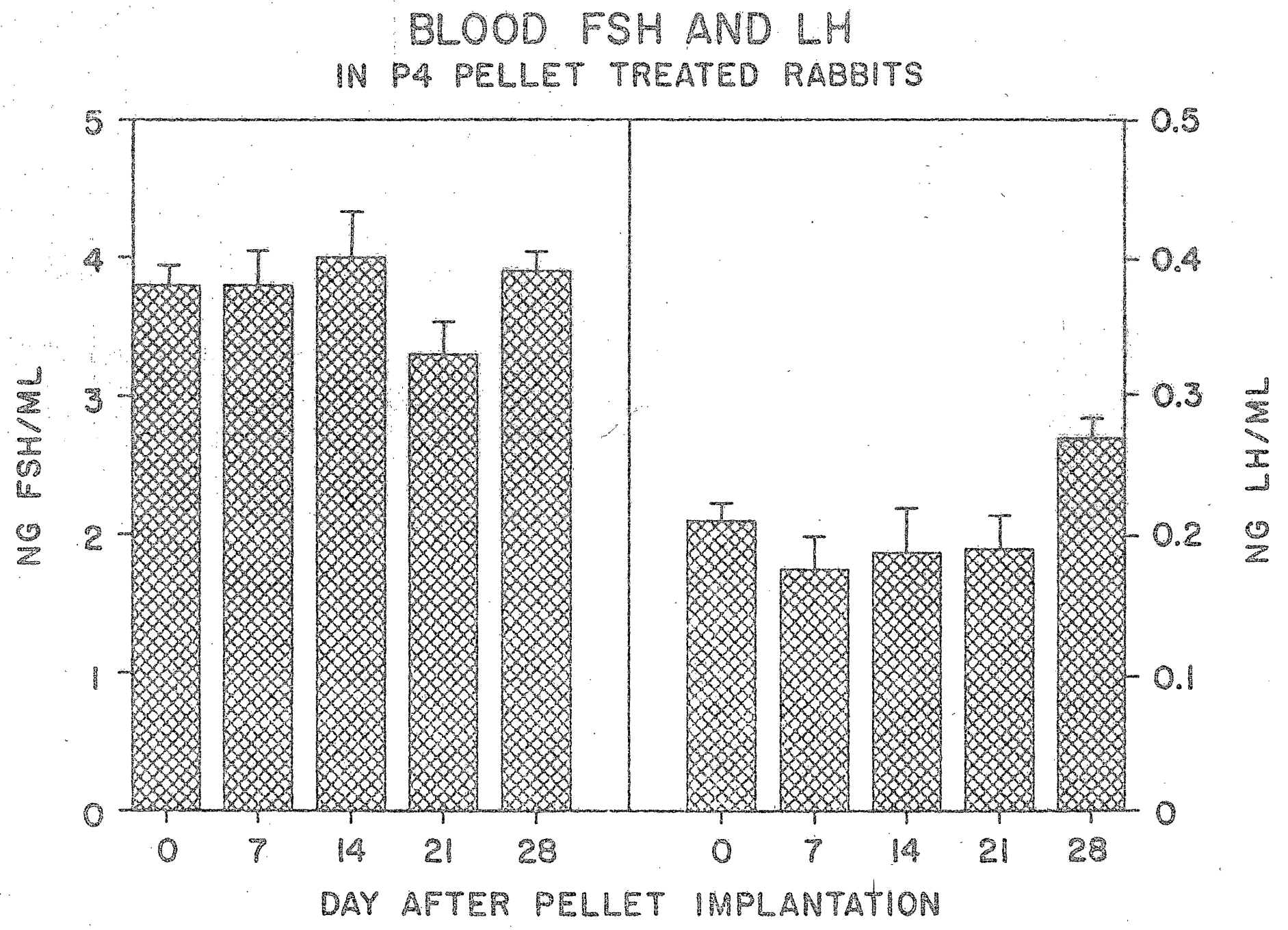
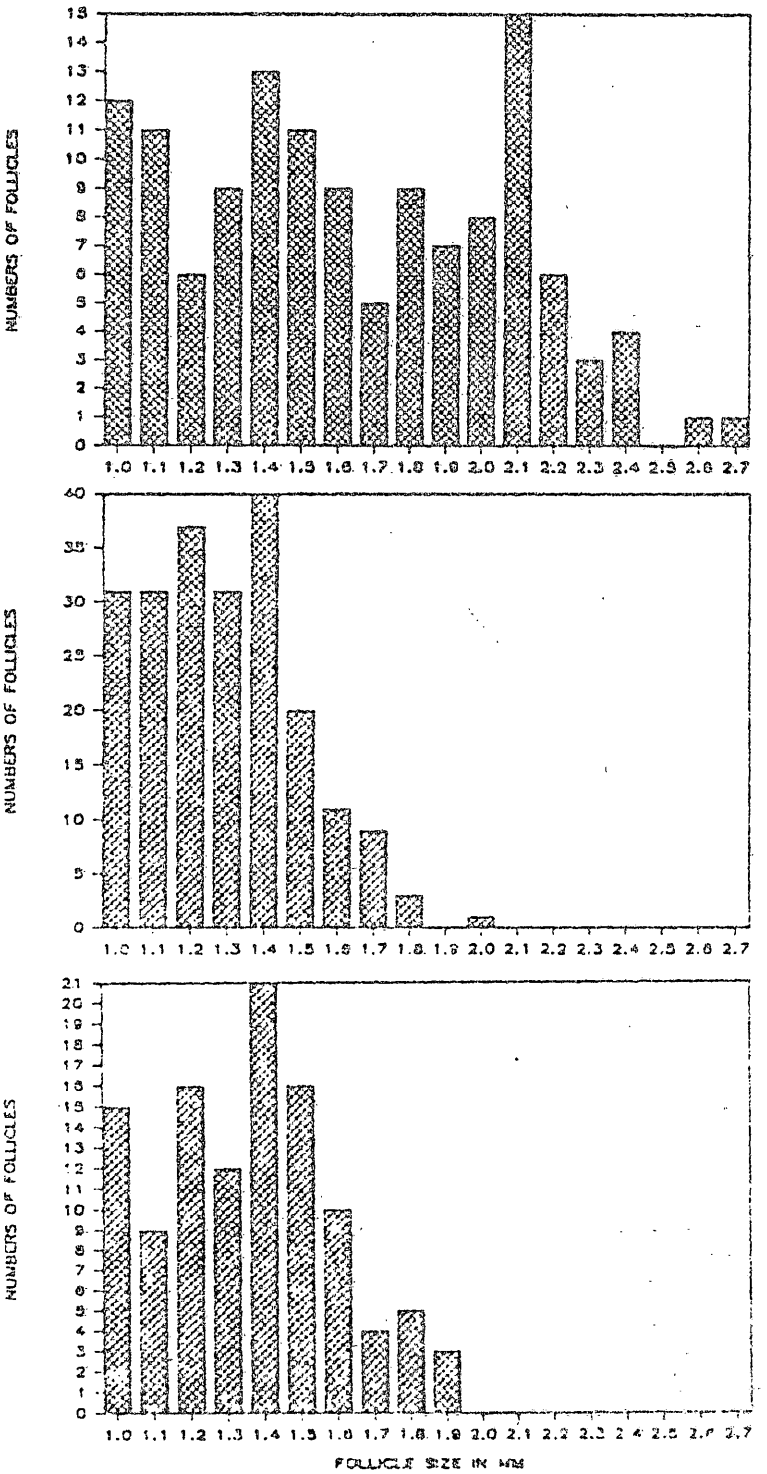

BLOOD FSH AND LH
IN P4 PELLET TREATED RABBITS
NG FSH/ML
NG LH/ML
0
1
2
3
4
5
0
0.1
0.2
0.3
0.4
0.5
0
7
14
21
28
0
7
14
21
28
DAY AFTER PELLET IMPLANTATION

Figure 22. Comparison of the Distribution of Follicle Sizes in the Ovaries of Non-Pregnant (upper panel), Pregnant (center panel) and Progesterone Treated, Non-Pregnant (lower panel) Rabbits.

On the ordinate are the number of follicles of various follicular diameters in millimeters (mm) on the abscissa. Note the similarity between the pattern of follicle sizes in the pregnant and progesterone treated animals. Both groups lack any follicles greater than 2.0 mm whereas these sizes are prevalent in the non-pregnant rabbits.


NUMBERS OF FOLLICLES
NUMBERS OF FOLLICLES
NUMBERS OF FOLLICLES
FOLLICLE SIZE IN MM

Characterization of the Size Distribution of Ovarian Follicles After Progesterone Withdrawal

In order to verify that progesterone is the regulatory agent in inhibiting follicular growth and development, another investigation was undertaken. Eight progesterone pellets were implanted in normal, non-pregnant does for fourteen days. Then progesterone pellets were removed from all the animals. Half the does were sacrificed at 7 days after withdrawal of progesterone and the other half were sacrificed at 14 days after removal of progesterone pellets. At the time of sacrifice, ovaries were removed, follicles were collected and external diameters were measured. Each follicle was assayed for estradiol and testosterone content.

Figures 23 and 24 demonstrate that following 14 days progesterone treatment and 7 days after progesterone withdrawal, the follicles that were present were all less than 1.7 mm. Furthermore, estradiol (Figure 23) and testosterone secretion (Figure 24) continued to be suppressed in comparison to follicular steroid secretion in non-pregnant animals (Figures 4 and 9). Apparently, even 7 days after removal of progesterone pellets, progesterone continued to have an inhibitory effect on follicular growth and steroid secretion.

However, follicles obtained from animals which had undergone progesterone pellet treatment for 14 days and examined 14 days following progesterone removal, showed that large follicles ($\geq$ 1.7 mm) were once again present (Figures 25 and 26). In addition, these follicles contained elevated levels of estradiol

(Figure 22) and testosterone (Figure 24), mimicking values found in non-pregnant animals (Figures 4 and 9). These results indicate that the inhibition of folliculogenesis and steroidogenesis caused by elevated serum levels of progesterone, are reversible. Fourteen days after progesterone pellet removal, follicular growth, and steroid secretion were comparable to those found in normal, intact, non-pregnant rabbits.

Figure 23. The Total Estrogen Content of Individual Follicles Isolated from Rabbit Ovaries that had Received Fourteen Days Progesterone Treatment Followed by Seven Days Progesterone Withdrawal.

On the ordinate are the total estrogen content per individual follicle in picograms. On the abscissa are the external diameters of the follicles in millimeters (mm). Follicles less than 1 mm in diameter were not collected.

FOLLICULAR ESTRADIOL

14 D PROG TREATMENT; 7 D WITHDRAWAL

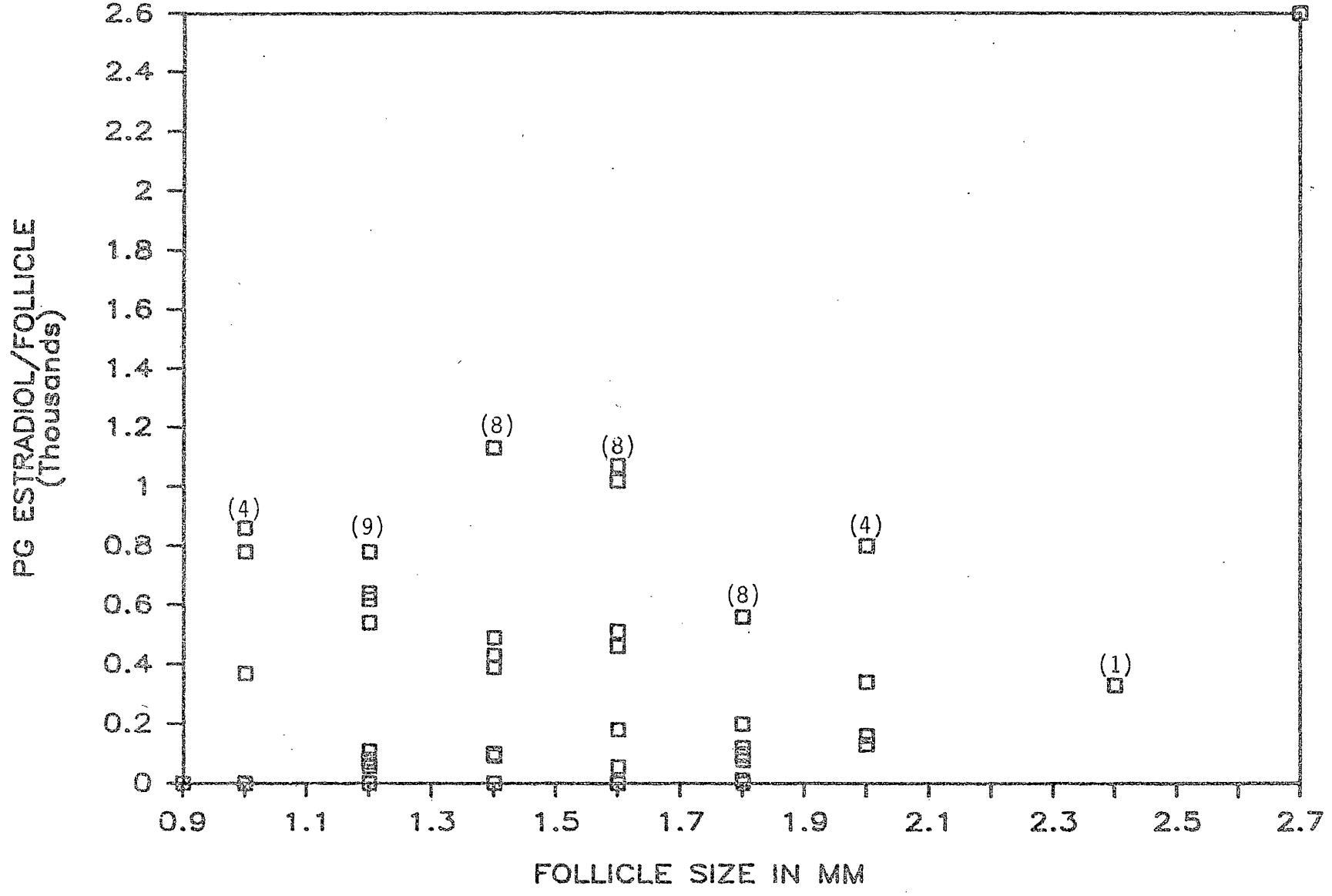


Figure 24. The Total Testosterone Content of Individual Follicles Isolated from Rabbit Ovaries that had Received Fourteen Days Progesterone Treatment Followed by Seven Days Progesterone Withdrawal.

On the ordinate are the total testosterone content per individual follicle in picograms. On the abscissa are the external diameters of the follicles in millimeters (mm). Follicles less than 1 mm in diameter were not collected.

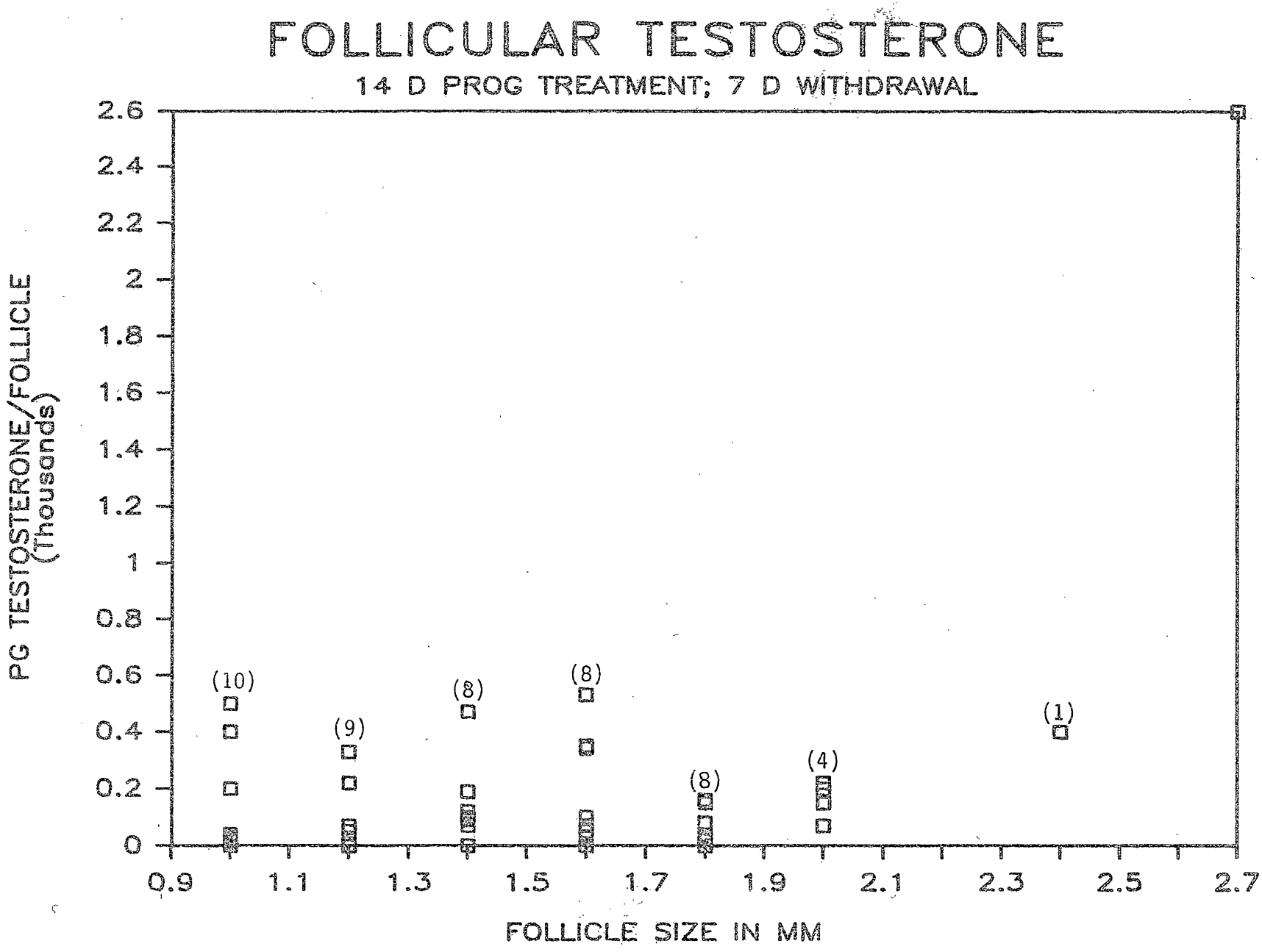

FOLLICULAR TESTOSTERONE
14 D PROG TREATMENT; 7 D WITHDRAWAL
PG TESTOSTERONE/FOLLICLE (Thousands)
2.6
2.4
2.2
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
(10)
(9)
(8)
(8)
(8)
(4)
(1)
0.9
1.1
1.3
1.5
1.7
1.9
2.1
2.3
2.5
2.7
FOLLICLE SIZE IN MM

Figure 25. The Total Estrogen Content of Individual Follicles Isolated from Rabbit Ovaries that had Received Fourteen Days Progesterone Treatment Followed by Fourteen Days Progesterone Withdrawal.

On the ordinate are the total estrogen content per individual follicle in picograms. On the abscissa are the external diameters of the follicles in millimeters (mm). Follicles less than 1 mm in diameter were not collected.

FOLLICULAR ESTRADIOL

14 D PROG TREATMENT; 14 D WITHDRAWAL

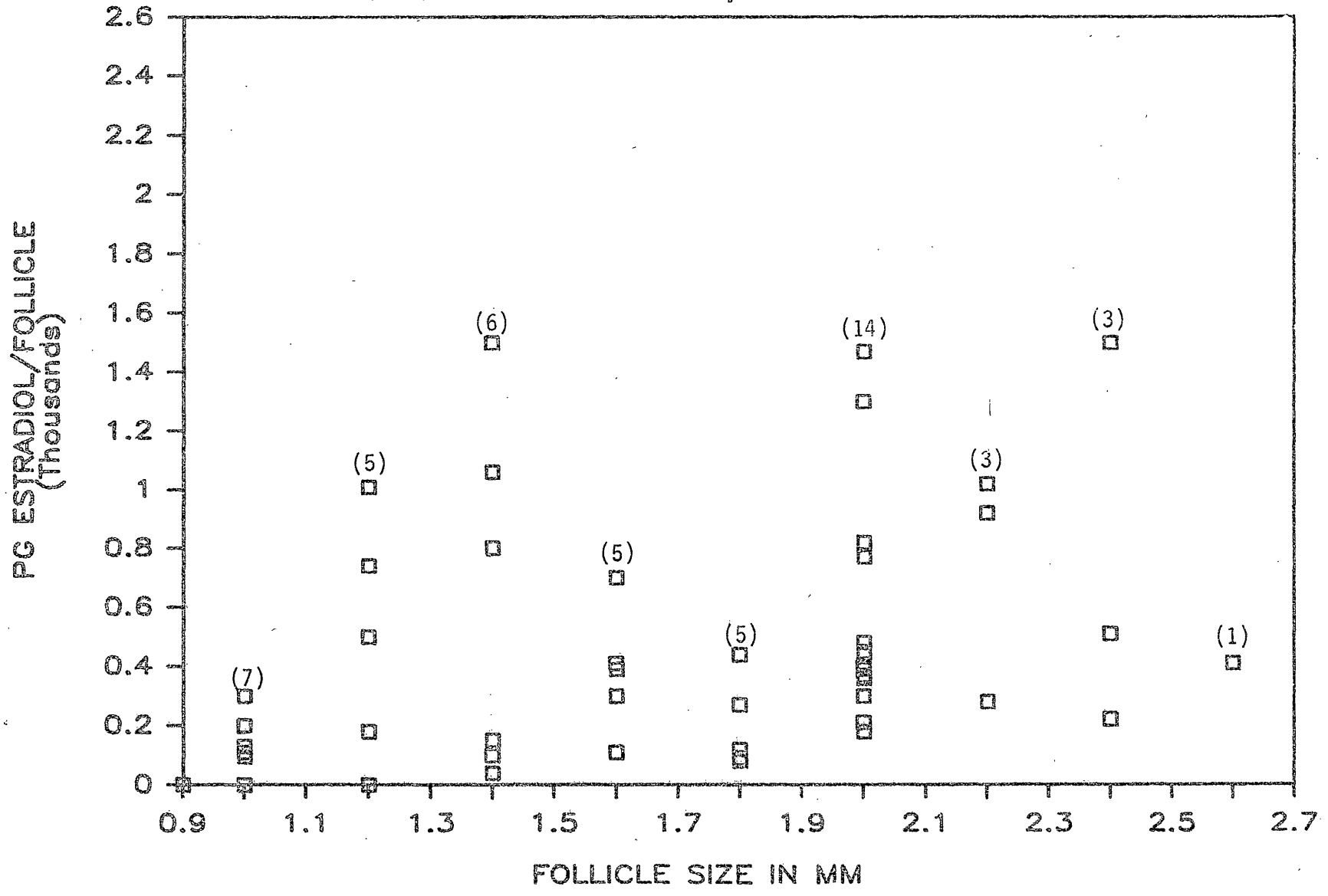
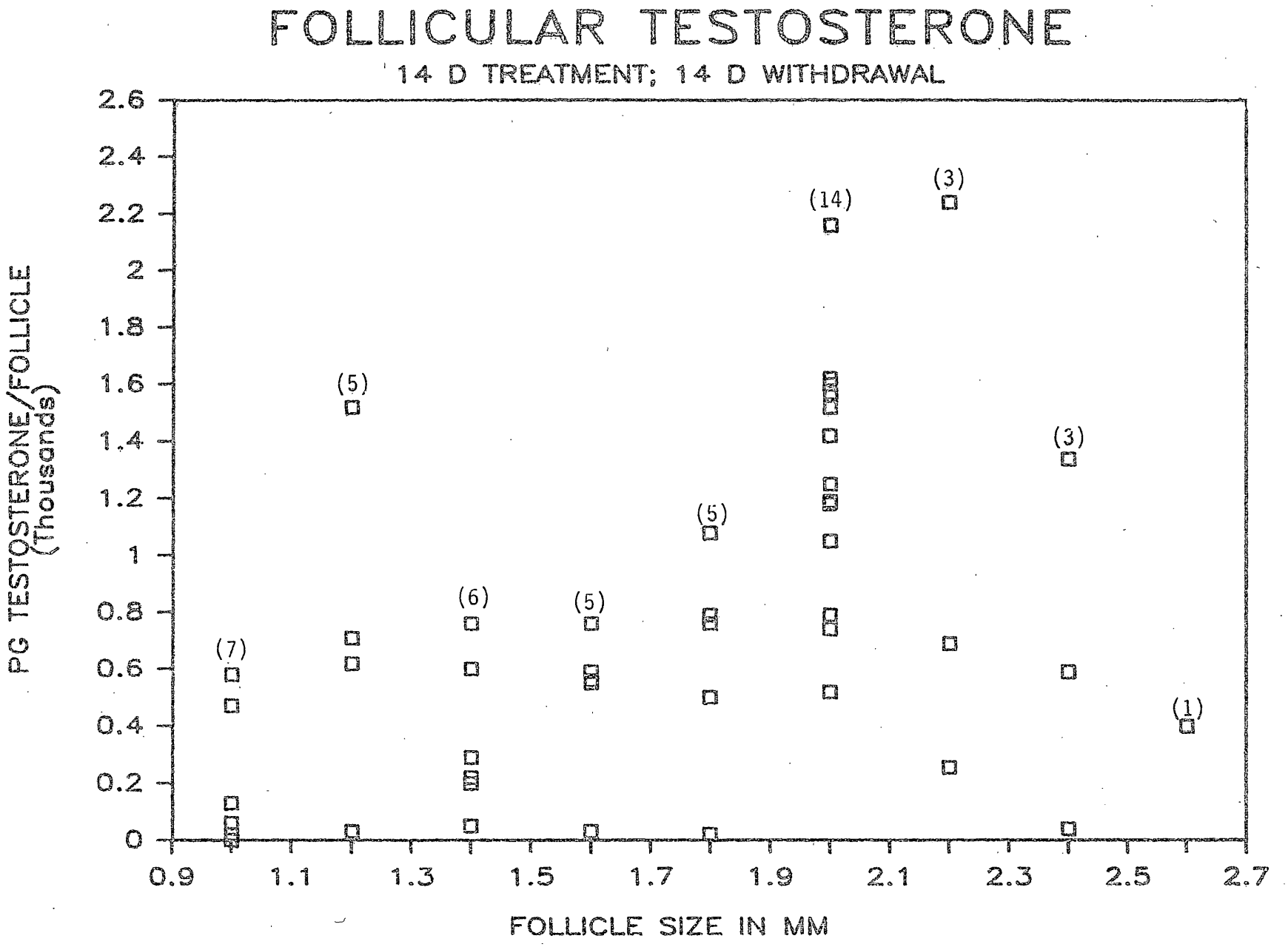


Figure 26. The Total Testosterone Content of Individual Follicles Isolated from Rabbit Ovaries that had Received Fourteen Days Progesterone Treatment Followed by Fourteen Days Progesterone Withdrawal.

On the ordinate are the total testosterone content per individual follicle in picograms. On the abscissa are the external diameters of the follicles in millimeters (mm). Follicles less than 1 mm in diameter were not collected.


FOLLICULAR TESTOSTERONE
14 D TREATMENT; 14 D WITHDRAWAL
PG TESTOSTERONE/FOLLICLE (Thousands)
2.6
2.4
2.2
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
(7)
(5)
(6)
(5)
(5)
(14)
(3)
(3)
(1)
0.9
1.1
1.3
1.5
1.7
1.9
2.1
2.3
2.5
2.7
FOLLICLE SIZE IN MM

Discussion

In the rabbit, estradiol has now been recognized as the primary luteotropic hormone because it can maintain functional corpora lutea after hypophysectomy (Robson, 1937; Hilliard, Spies, et al., 1969). The critical dependence of the rabbit corpus luteum on estrogen is clearly demonstrated by the rapid decline of serum progesterone after withdrawal of estradiol from the circulation (Holt, Keyes et al. 1975) or after X-ray destruction of follicles (Keyes and Nalbandov, 1967; Keyes and Armstron, 1968). Furthermore, replacement of estradiol via an silastic implant containing 17β-estradiol promptly restored serum progesterone levels to normal (Keyes, Yuh, et al. 1979).

It is now well established that progesterone, secreted by the rabbit corpus luteum, is absolutely essential for the maintanence of pregnancy (Allen and Corner, 1929; Wu and Allen, 1958). However, the rabbit corpus luteum does not synthesize estrogen (Mills and Savard, 1973) nor does it appear to have any detectable aromatase activity (Elbaum and Keyes, 1973). Therefore, the corpus luteum develops an obligatory dependence upon estradiol secreted by the ovarian follicles (Keyes and Nalbandov, 1967; Keyes and Armstrong, 1968), by day 6 post coitum (Miller and Keyes, 1978). Since these Graafian follicles are the principal source of estrogen, sufficient number of steroidogenically active follicles need to be present in order to maintain proper luteal function. However, following mating, most of the large follicles are lost to the ovulatory process. Therefore, in order to

maintain secretion of the luteotropic estrogen, new follicular growth must occur in the postovulatory rabbit.

During pregnancy, follicular growth has to occur in a hormonal milieu rich in progesterone, due to the presence of the actively secreting corpora lutea. Previous investigators have presented data suggesting an inhibitory role of progesterone on follicular development (Jesel, 1970; Buffler, 1974; Goodman, 1977). However, others have reported that progesterone does not inhibit gonadotropin-induced follicular maturation in the monkey ovary (Zeleznik and Resko, 1980) nor does exogenous progesterone have any effect on rat ovarian morphology (Richards, 1975; Saiduddin and Zassenhaues, 1978). Therefore, the regulatory action of progesterone on the control of ovarian follicular development has not yet been fully defined.

Mills and Copland (1981) had proposed that elevated levels of progesterone would be expected to suppress circulating gonadotropins. These investigators had also proposed that the postovulatory FSH surge initiated new follicular growth and that these antral follicles would develop rapidly and secrete the luteotropic estrogen necessary for the continuation of pregnancy. However, when this postovulatory FSH surge was blocked utilizing porcine follicular fluid containing inhibin, there was no loss of pregnancy. Therefore, even in the absence of the postovulatory FSH surge, follicular growth continued and pregnancy was maintained. Consequently, factors other than the postovulatory FSH secretion must play a regulatory role in controlling follicular growth and development in the pregnant rabbit.

The role of progesterone in the regulation of follicular growth and development has not been fully elucidated in an animal like the rabbit in which the corpus luteum is totally dependent on follicular estrogen. The experiments reported in this thesis were undertaken to determine if follicular growth can occur throughout pregnancy and other periods during which serum levels of progesterone are elevated. Furthermore, this study was the first to investigate the role of progesterone as a major regulatory factor in the growth and development of ovarian follicles in a species which shows induced ovulation.

Follicular Growth in the Pregnant Rabbit Ovary

Follicular growth in the pregnant rabbit was examined by measuring the external diameters of all follicles that were greater than 1 mm in diameter and present through weeks 1, 2, 3 and 4 of pregnancy. Data indicate that large follicles ranging from 1.0 mm up to 2.5 mm are present in the week 0 (preovulatory) rabbits but by week 1 of pregnancy, many of these large follicles had been lost due to ovulation. While the large follicles (> 1.7 mm) were depleted by week 1, many follicles in the medium and small size range (1.0 mm-1.7 mm) continued to be present. This is in concordance with Asami's (1920) finding that by Day 7 postcoitum, a normal follicle population was again present in the postovulatory rabbit. While, throughout the remainder of pregnancy (weeks 2, 3 and 4), follicles ranging from 1.0 mm to 1.7 mm were consistently found, the large follicles (> 1.7 mm) were completely absent. Therefore, in the rabbit, follicles are continuously present throughout the entire period of pregnancy

up to the time of parturition. Since it has been estimated that between 2 and 3 weeks are required for the turnover of follicles in the pregnant rabbit and since follicles were present throughout the gestation period, follicular growth and turnover must continue throughout pregnancy.

Steroid Content of Ovarian Follicles in the Pregnant Rabbit Ovary

Ovarian follicles $\geq$ 1.0 mm in external diameter were collected from rabbit ovaries at week 0, 1, 2, 3 and 4 of pregnancy. To determine the steroidogenic capability of follicles during pregnancy, each individual follicle was assayed for total content of estradiol and testosterone. The steroid content of large follicles removed from week 0 (non-pregnant) rabbits was high while smaller follicles generally had lower steroid contents. Such direct correlation between size of follicle and steroid content had been demonstrated for the human (Sanyal et al., 1974), rhesus monkey (Clark et al., 1979) and sheep ovaries (Hay and Moor, 1978).

In contrast to the nonpregnant animals, follicles obtained from 1 week pregnant rabbit ovaries, displayed a low concentration of estradiol and testosterone. In addition to lacking large follicles (> 1.7 mm), the pregnant animals did not demonstrate any correlation between size and steroid content of large follicles. In other words, there were examples of larger follicles with lower steroid content and even some small follicles which contained high concentrations of one or both of the steroids. As pregnancy continued, follicles collected at weeks 2, 3 and 4 of pregnancy continued to show depressed levels of estradiol and testosterone,

in comparison to follicular steroid content of nonpregnant animals. Therefore, follicles removed from various reproductive states (nonpregnant vs. pregnant) display different steroid secretion profiles. These results suggest that the longer the period of time the follicles are located in close proximity to actively secreting corpora lutea, the more depressed is their steroid secretion.

Serum Gonadotropins in the Pregnant Rabbit

Blood samples collected from the same group of does immediately before mating and on day 14 of pregnancy were assayed for LH and FSH concentrations. Both of these gonadotropins are important in initiating follicular growth and steroidogenesis (Channing and Tsafriri, 1977). This quantification of serum levels of gonadotropins was preformed in order to determine whether the high levels of circulating progesterone could indeed be exerting an inhibitory action of folliculogenesis and hormonogenesis via the hypothalamic-pituitary axis. If so, there should be a suppression of serum levels of LH and FSH in the pregnant animals. Suprisingly, not only was there not a decrease in either gonadotropins, but there was actually a significant increase in the levels of FSH measured in the 14 day pregnant animals, when compared to levels in the same group of animals before mating (day 0). It can be concluded that the elevated levels of circulating progesterone are exerting a direct inhibition on follicular growth and turnover; progesterone's suppressive actions are not mediated through inhibition of gonadotropic secretion.

Follicle Growth in the Progesterone Treated Rabbit Ovary

This next investigation was performed to prove that progesterone, and not some other steroidal or non-steroidal factor secreted during pregnancy, was the regulatory agent inhibiting folliculogenesis during pregnancy. After confirmation that eight pellets of crystallized progesterone elevated serum levels of progesterone to approximately 5-8 ng/ml, (this is below the value measured during pregnancy but it is sufficiently above what is usually found in a nonpregnant animal), normal intact does were implanted with eight progesterone pellets per rabbit. This treatment resulted in serum levels of progesterone being elevated to nearly the levels found in pregnant animals.

During the continuous progesterone treatment, follicles were removed at 0, 1, 2, 3 and 4 weeks. These time periods were specifically chosen to provide a suitable comparison of results obtained from pregnant animals at the same intervals. Measurement of follicular diameters again showed that large follicles (> 1.7 mm) were missing in the progesterone treated rabbits, although large follicles were present in the untreated animals (week 0). Therefore, progesterone treatment of unmated rabbits mimicked the inhibition of follicular growth observed during pregnancy. Furthermore, follicular steroid secretion seemed to exhibit the same suppression as was seen in pregnant animals from week 1 through week 4 of pregnancy. Both estradiol and testosterone never attained the serum concentrations found in nonpregnant, untreated rabbits. These data provide evidence that increased levels of circulating progesterone, although not elevated to those levels found during

pregnancy, are still sufficient to arrest the growth and development of follicles and their steroid production.

Follicular Growth in Rabbits Undergoing Progesterone Withdrawal

Having established that progesterone was the principal luteal factor suppressing follicular growth and steroidogenesis during pregnancy, it next became important to show that this inhibitory action was reversible. Nonpregnant does were implanted with progesterone pellets for fourteen days after which all eight pellets were completely removed. The does were then sacrificed 7 days or 14 days after pellet removal. Animals sacrificed at 7 days after progesterone withdrawal, displayed follicles that were all < 1.7 mm in diameter. Furthermore, in these animals, estradiol and testosterone secretion continued to be below what was found in nonpregnant, untreated animals. Apparently, at 7 days after pellet removal, the inhibitory effect on folliculogenesis and steroid secretion was sustained. However, does that were sacrificed at fourteen days after progesterone pellet removal showed that large follicles (> 1.7 mm) ranging up to 2.5 mm again populated the ovary. These follicle sizes were very similar to those follicles found in nonpregnant rabbits. Furthermore, both estradiol and testosterone concentrations were once again elevated to the levels indicative of nonpregnant animals. All these results provide evidence to indicate that progesterones plays a principal role in suppressing folliculogenesis and hormonogenesis. However, the inhibition exerted by progesterone is completely reversible. Removal of progesterone implants caused a reinitiation of

follicular growth and development and normal secretion of estradiol and testosterone.

Summary

Investigation of follicular dynamics in nonpregnant, pregnant and progesterone-treated rabbits clearly demonstrated that follicular growth and turnover continue to occur, even during reproductive states where serum levels of progesterone are elevated. While follicular growth continues, the follicles in pregnant and progesterone treated animals never attain the size found in nonpregnant animals; the progesterone is clearly inhibiting follicles from reaching their maximum size. Furthermore, the estradiol and testosterone content of follicles collected from pregnant and progesterone treated rabbits never reach the concentrations measured in follicles from nonpregnant, untreated animals. Characteristics of follicular growth, turnover and steroid content are similar in pregnant and progesterone treated rabbits, and different from follicles in nonpregnant animals. Taken together, these lines of evidence point to progesterone as the principal causative agent in mediating inhibition on folliculogenesis and steroidogenesis. Nevertheless, progesterone, although inhibitory in action, still allows for sufficient follicular growth, turnover and steroidogenesis to maintain proper luteal function and sustain a normal pregnancy. In addition, progesterone's role in modulating follicular development is completely transient. Once the levels of progesterone are returned to normal, all the suppressive actions of this hormone are reversed, and normal follicular growth and development is reinitiated.

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