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RABBIT

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The effects of suckling on several ovarian characteristics were studied in postpartum rabbits. Ovarian weight was found to be significantly greater in rabbits suckled for twelve days than in nonsuckled rabbits autopsied at a similar interval postpartum or in a third group autopsied at parturition. There were no significant differences between groups in ovarian content or concentration of progestogens (i. e., progesterone and  $20\alpha$ -hydroxypregn-4-en-3-one). It was concluded that the observed increase in ovarian weight in suckled does was due to an ovarian characteristic not measured in this study. Spontaneous ovulation occurred in two out of six does in the suckled group but not in does in either of the other two experimental groups.

Some Ovarian Characteristics  
of the Postpartum Rabbit

by

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## SOME OVARIAN CHARACTERISTICS OF THE POSTPARTUM RABBIT

### INTRODUCTION AND REVIEW OF LITERATURE

One potentially lucrative opportunity for the producer of domestic animals to increase profits lies in the improvement of reproductive efficiency during the postpartum period, for in all economically important species this interval is characterized by prolonged periods of ovarian quiescence, poorly synchronized occurrence of cyclic events which are essential to conception, and low fertility levels in general, as a result of these anomalies. Consequently, the postpartum female has been the object of numerous scientific investigations in recent years, most of which have been aimed at gaining a better understanding of her reproductive characteristics in hopes that by comparing these to the reproductive characteristics exhibited by the estrual female, some method of improving postpartum reproductive performance might be indicated. As a result of these studies it has been found that two of the most important factors contributing to postpartum reproductive inefficiency are a prolonged period to first ovulation and an increase in the incidence of ovulation without simultaneous occurrence of behavioral estrus. In response to these findings much of the subsequent work was conducted with the goal of determining whether the interval to first ovulation with estrus could be shortened, and if it could, whether

mating at this time would result in economically acceptable levels of conception and embryonic survival.

### Reproductive Characteristics of the Female in Advanced Pregnancy

The reproductive characteristics of the postparturient female are, in part, a reflection of conditions present during late pregnancy, therefore, this stage of gestation has received considerable investigative attention.

It has been found, during pregnancy as during the estrous cycle, that progesterone appears to be the dominant hormone even though considerable interspecies variation exists in relation to the source of progestogens as well as in relation to the pattern of their production throughout pregnancy. In cattle, the corpus luteum is the major source of progesterone as well as its metabolite,  $20\beta$ -hydroxypregn-4-en-3-one (Stormshak and Erb, 1961). In the rabbit, however, progesterone is produced by both the luteal tissue and the ovarian interstitial tissue while the progesterone metabolite, which in this species is  $20\alpha$ -hydroxypregn-4-en-3-one, is produced primarily by the interstitial tissue. (Hilliard, Archibald and Sawyer, 1963; Hilliard et al., 1968). As mentioned previously, patterns of progesterone production during pregnancy also vary between species. In the cow, levels of peripheral plasma progesterone fluctuate until approximately 250 days of pregnancy at which time a relatively rapid decline

begins resulting in rather low levels being present at parturition (Gomes and Erb, 1965). On the other hand, analogous measurements in the rabbit reveal a slow but steady rise to a peak at mid-pregnancy followed by a gradual decline during the second half of gestation (Mikhail, Noall and Allen, 1961; Hafez, Tsutsumi and Kahn, 1965). It is assumed that in addition to acting on the uterus to maintain pregnancy, progesterone also depresses ovarian activity indirectly through its inhibitory effect on gonadotropin secretion from the anterior pituitary gland, at least in some species (Labhsetwar, 1964; Hansel and Snook, 1970). This would certainly seem to be indicated in the case of the bovine, since in this species follicular development in the pregnant female declines as gestation progresses, and consequently is in a depressed state at parturition (Nalbandov and Casida, 1940; Labhsetwar, 1964). In the rabbit (Adams, 1964) and hamster (Greenwald, Keever and Grady, 1967), however, follicular development is greater during the prepartum period than during early gestation or the postpartum period. There is some indirect evidence that prepartum ovarian function could be caused by extrapituitary gonadotropins. The placenta of the human and the rat and the endometrium of the pregnant mare (Selye, 1933; Noble and Plunkett, 1955) are known to produce gonadotropins. In addition the rabbit placenta is suspected of being the source of a substance which is luteotropic (Chu, Lee and You, 1946; Stormshak and Casida, 1966). These facts suggest, but in no way prove

conclusively, that the prepartum ovarian function in the rabbit and hamster could be due to a gonadotropin-like substance produced by the placenta which is capable of stimulating follicular growth. This would seem to be the most feasible explanation for the prepartum ovarian activity in these species since by comparing their pituitary follicle stimulating hormone (FSH) levels with those present in the cow it is found that all three animals are relatively rich in this pituitary gonadotropin at parturition (Labhsetwar, 1964; Greenwald et al., 1967). This would seem to indicate a storage of FSH on the part of the pituitary, especially in the cow, but the possibility remains in rabbits and hamsters that the observed follicular development is, indeed, due to FSH released from the pituitary in late pregnancy and that the relatively high pituitary levels at parturition are simply an indication that the rate of FSH synthesis exceeds the rate of its release. If this did prove to be the case it would imply, of course, that complete progesterone inhibition of pituitary gonadotropin secretion does not occur in these species during advanced pregnancy. In general, pituitary luteinizing hormone (LH) is relatively low in the cow, the rabbit and the hamster at parturition (Labhsetwar, 1964; Saiduddin and Foote, 1964; Greenwald et al., 1967).

#### Reproductive Characteristics of the Postpartum Female

Following parturition, lactation is initiated and maintained by a marked increase in the content and secretory rate of pituitary prolactin

in all mammalian species (Holst and Turner, 1939; Meites and Turner, 1948a; Hurst and Turner, 1948). However, interspecies variation in pituitary gonadotropin levels and ovarian activity continue during lactation. For example in the cow, pituitary FSH levels decrease and pituitary LH levels increase during this period. (Labhsetwar et al., 1964; Saiduddin and Foote 1964). These changes reflect a pituitary release in the case of FSH and a pituitary accumulation in the case of LH, the results of which are a significant increase in follicular development as early as ten days after parturition. This development in turn proves to be in preparation for ovulation which occurs in the presence of lactation 40-50 days postpartum. (Casida and Venzke, 1936; Buch, Tyler and Casida, 1955). On the other hand, in the rabbit (Adams, 1964) and hamster (Greenwald et al., 1967) the pituitary FSH content continues to increase during lactation and is accompanied by concomitant low levels of pituitary LH. As a result, follicular atresia and luteolysis reduce the ovary to little more than interstitial tissue during lactation in the hamster (Greenwald et al., 1967) and in the rabbit, Adams (1964) has reported that the ovary ovulates significantly less during lactation than before parturition when injected with similar doses of LH, thereby suggesting that follicular development is also less in this species after parturition. These postpartum variations are deeply rooted in the evolutionary past and, more likely than not, have occurred because of the more basic differences present between

these species in relation to length of gestation, persistence of lactation, rapidity of development and inherent levels of fecundity.

Lactation in the female, characterizes the postpartum period in mammals, at which time it serves the purpose of providing sustenance for young delivered in such a stage of development that they are unable to survive without the special nutritive advantage provided by the mammary secretion. The process of milk secretion and removal entails the involvement of prolactin, adrenocorticotrophic hormone and growth hormone secreted chronically from the maternal anterior pituitary gland (Meites, 1965; Averill, 1966, Grosvenor, Krulich and McCann, 1968) in addition to acute release of oxytocin from the maternal posterior pituitary gland (Mizuno, Talwalker and Meites, 1967). These pituitary secretions are released in response to nervous stimulation provided by nursing of the young. Acute intensity of this stimulus, adjusted by controlling the number of nursing young, seems to have very little differential effect on the amount of the pituitary hormones released in rabbits (Meites and Turner, 1948b) or rats (Grosvenor, Krulich and McCann, 1968). However, chronic differences in the intensity of the suckling stimulus do lead to significant differences in pituitary prolactin content in rats (Mena and Grosvenor, 1968). As stated previously, nursing is the primary stimulus causing release from the pituitary of hormones involved in galactopoesis but this release can become conditioned, at least in the rat, so that it occurs in

response to various exteroceptive stimuli such as sight of the young alone even though no actual tactile contact between mother and young is allowed (Grosvenor, 1965; Moltz, Levin and Leon 1969). In addition, it has been found by Meites (1957) that reserpine, a drug which induces the release of large amounts of serotonin from the central nervous system (Shore, Silver and Brodie, 1955), can inhibit the release of prolactin from the anterior pituitary in rabbits. Grosvenor and Mena (1967) have reported that various optic and auditory stimuli are also capable of inhibiting normal prolactin release as well as the suckling-induced release of oxytocin in the rat. These experimental findings clearly indicate a neural involvement in the release of galactopoietic hormones from the pituitary gland in response to suckling in these species.

Considering the extensive effects of suckling upon the endocrine system of the lactating female it is perhaps not too surprising to find that the reproductive system during the postpartum period, which of course depends largely on the same endocrine system to function properly, is profoundly affected by concurrent lactation. The effects of lactation upon postpartum reproductive efficiency in the bovine have been especially well studied. When compared to nonsuckling in this species, the stimulus of suckling has been found: (a) to increase the length of the period from parturition to first estrus, (Clapp, 1937; Saiduddin, et al., 1967; and Oxenreider, 1968) due, at least in part, to an

increased incidence in the occurrence of ovulation not accompanied by behavioral estrus (Casida and Venzke, 1936; Kidder, Barret and Casida, 1952; Marrow et al., 1966); (b) to increase pituitary FSH content (Saiduddin et al., 1967; Wagner, Saatman and Hansel, 1969); (c) to have no effect on pituitary LH content (Saiduddin et al., 1967); Wagner et al., 1969); and (d) to decrease pituitary prolactin activity (Clapp, 1937) and more recently by investigators at the University of Wisconsin (1968). In addition, suckling during the postpartum period in cattle has been reported to hasten the involution of the uterus back to its non-gravid size in at least two studies (U. of Wisconsin, 1968) although it has been reported to have no effect on the same characteristic in another study (Oxenreider, 1968). In support of Oxenreider's work, Wagner and Hansel (1969) found that nursed cows did not differ in their degree of uterine involution when compared to cows milked twice daily. In swine, however, Peters, First and Casida (1969) found that suckled sows had depressed uterine weights when compared to mammillectomized sows with pigs present. These conflicting reports make a conclusive statement regarding the effect of suckling on uterine involution impossible and indicate a need for further investigation of the subject. Wiltbank and Cook (1958) found that reproductive efficiency as measured by length of the intervals; from parturition to first postpartum ovulation, from parturition to first postpartum estrus and from parturition to conception, was considerably better in cows

milked twice daily than in cows nursing calves. There is evidence that this observed difference is more likely due to the frequency of milk removal than to the presence of the calf, since workers at the University of Wisconsin (1968) found no difference in the length of the interval from parturition to first succeeding estrus when suckled cows were compared with cows milked four times daily. There was a significant difference, however, between both of these groups and a third group of cows milked only twice daily. In addition, it has been found in the rat that suckling for five or thirty minutes in dams which had been isolated from their litters for eight and one-half hours resulted in essentially the same amount of pituitary prolactin depletion. If the mothers were isolated for 16 hours prior to suckling it was found that neither five nor thirty minutes of nursing stimulation were able to effect a decrease in pituitary prolactin content. These findings led the authors to conclude that, in the rat, the mechanism involved in prolactin release in response to suckling operates efficiently only if the stimulus is applied periodically at short intervals (Grosvenor, Mena and Schaeffgen, 1967).

Recurrent application of the suckling stimulus in the normally lactating female implies, of course, that oxytocin is released periodically from the posterior pituitary since this substance is known to be necessary for milk ejection (Benson and Cowie, 1956). Experimental evidence indicates that there are considerable differences among

species in respect to pituitary and gonadal responses caused by administration of exogenous oxytocin. In normal immature rats or in mature rats with hypothalamic lesions, infusion of oxytocin into the third ventricle causes premature vaginal canalization and increased weight of the reproductive organs, suggesting stimulation of FSH and LH release (Corbin and Schottellius, 1961). In addition Haun (1966) found that infusion of oxytocin into the third ventricle of adult rats caused pseudpregnancy on any day of the cycle except on the day of proestrus and Martini et al. (1959) have reported that intravenous injection of oxytocin induces a significant rise in urinary gonadotropin output in normal female rabbits. In contrast to these data are findings by Brinkley and Nalbandov (1963) that oxytocin injections in rats were unable to affect the life span of induced corpora lutea suggesting that in this experiment stimulation of prolactin release did not occur since prolactin is luteotropic in the rat (Turner, 1968). In addition oxytocin did not induce LH secretion in mature female rats as measured by ovarian ascorbic acid depletion (Guiliani et al. 1961). It has also been found that oxytocin injected subcutaneously within the interval from 4.25 to 19 minutes prior to mating in rabbits inhibits the coitus-induced release of LH (Brinkley and Nalbandov, 1963). In swine, oxytocin injected intramuscularly every two hours from day one postpartum until autopsy on day seven postpartum depresses follicular fluid weight and number of large follicles in sows if young are present but not if

young are removed (Peters et al., 1969). One of the primary findings supporting the hypothesis that oxytocin was capable of stimulating the release of anterior pituitary hormones was the fact that milk secretion was maintained significantly longer in oxytocin injected rats than in saline injected rats when litters were removed from all dams on day four postpartum (Benson and Folley, 1957). In 1961, however, Meites and Hopkins demonstrated that the favorable effects of oxytocin in respect to maintenance of secretory activity and retardation of mammary involution in rats were not exerted via the hypothalamus since the oxytocic effect was observed to occur in hypophysectomized as well as intact animals. The effects of exogenous oxytocin administration have been especially well studied in the bovine where it has been established that oxytocin injections administered chronically shortly postestrus cause a marked shortening of the estrous cycle (Armstrong and Hansel, 1959; Hansel and Wagner, 1960; Anderson, Bowerman and Melampy, 1965). Armstrong and Hansel (1959) concluded that this oxytocin-induced shortening of the diestral period was caused by inhibition of normal corpus luteum function possibly due to interference by oxytocin with the secretion of a luteotropic hormone from the anterior pituitary. Subsequently Donaldson, Hansel and Van Vleck (1965) presented evidence that bovine luteinizing hormone is the luteotropic hormone in cattle and that concurrent administration of purified bovine LH or human chorionic gonadotropin

(HCG) causes a complete reversal of oxytocin-induced luteal regression. In further clarification of the mechanism of this regression it was shown that the presence of the uterus was essential for expression of the effect in cattle (Hansel and Wagner, 1960; Anderson, Bowerman and Melampy, 1965). The corpus luteum of the bovine seems to depend on LH stimulation for normal growth and function (Donaldson et al., 1965; Hansel and Seifart, 1967) and it may be that premature regression of the corpus luteum following chronic oxytocin administration early in the estrous cycle is the result of an inhibition of LH secretion or release from the anterior pituitary gland. There is, however, another school of thought with considerable experimental support which postulated that regression of the corpus luteum in the cow by the injection of oxytocin early in the cycle is caused by a direct action of oxytocin on the uterus (Anderson, Bland, and Melampy, 1969).

Studies on the effect of the stimulus of nursing, which are of course primarily studies on the effect of the endogenous release of prolactin and oxytocin have been conducted in many species. The present experiment was conducted as the result of observing a difference in the size of ovaries removed from postpartum rabbits while conducting a preliminary investigation, on the effects of suckling upon the growth rate of induced corpora lutea. It was noted in a limited number of animals that, although there appeared to be no effect

of suckling upon the induced corpora lutea, ovaries removed from suckled does seemed markedly heavier than ovaries removed from nonsuckled does. In light of the fact that ovaries in the rabbit are known to increase in weight throughout pregnancy (Hafez, Tsutsumi, Kahn, 1965) it was postulated that this difference could be due to ovarian hypertrophy in the suckled does, to ovarian atrophy in the nonsuckled does or to a combination of both processes. Consequently, in addition to the nonsuckled and suckled groups the experimental design also included a group autopsied at parturition to provide data which would allow conclusions to be drawn concerning the mechanism responsible for the expected difference in ovarian weight, and it was also proposed that the hormone content of the ovaries from all three groups would be investigated to determine if any correlations existed between this characteristic and ovarian weight.

## MATERIALS AND METHODS

Eighteen primiparous Dutch Belted rabbits were assigned in replicate to one of three groups of six does each. The groups and the treatments applied were as follows: Group I, controls, no treatment and autopsy on day one postpartum; Group II, nonsuckled, removal of the young at parturition and autopsy on day 12 postpartum; Group III, suckled, maintenance of the litter at eight young with unlimited access to these young provided the mother and autopsy on day 12 postpartum. The litters were maintained at the previously determined number of eight by immediate removal of excess young or by supplementation of a deficient litter with young kindled by rabbits in the control or nonsuckled groups as soon as these were available. In no case were more than eight or less than six young present in any litter and in the case of deficient litters the condition never prevailed for more than 48 hours. Very little difficulty was encountered in getting does in the suckled group to accept foster young. Group I was included to provide data on ovarian characteristics in the rabbit on day one postpartum and groups II and III were designed to investigate the effects of suckling on ovarian characteristics in the rabbit on day 12 postpartum. Throughout the experimental period the does were housed individually in wire cages until shortly before parturition at which time they were moved to maternity cages which were enclosed on three sides and afforded

a view of neighboring rabbits across an alleyway.

Immediately following autopsy the ovaries were removed and weighed to the nearest one-tenth milligram. The corpora albicantia (CA) were dissected out of the ovarian stromal tissue of rabbits in the control group (average CA weight of 12.2 mg) but not from the ovaries of rabbits in the nonsuckled or suckled groups. The small size of the corpora albicantia in the ovaries of rabbits in these latter two groups (average CA weight approximately 3.0 mg) by day 12 postpartum made location and dissection very difficult and it was decided that less variability would result by allowing them to remain than would be produced due to loss of follicular fluid and stromal tissue if the dissection were attempted. The ovaries of does in the control group were weighed again following removal of corpora albicantia. Ovaries from rabbits in all groups were sliced and stored in 10 ml of 95 percent ethanol until analyzed for progestogens.

Ovarian tissue was analyzed for progesterone and  $20\alpha$ -hydroxy- $20\alpha$ -pregn-4-en-3-one. Progesterone-4- $^{14}$ C and  $20\alpha$ -hydroxypregn-4-en-3-one-1, 2- $^3$ H were added to each sample to correct for losses incurred during extraction and purification. Ovarian tissue was homogenized in 95 percent ethanol and filtered. The filtrate was dried under vacuum and further purified by column chromatography as described by Stormshak et al. (1963). Final purification of samples was made by use of thin layer chromatography (E. Merck, silica gel F-254,

preparative, with inorganic fluorescent indicator). The chromatogram was washed in a chromatography tank containing chloroform:methanol (50:50 V/V) for one hour and then dried for 30 minutes at 80° - 90° C before spotting the entire sample on the plate. Chromatograms were allowed to develop in one dimension for two hours in a solvent system of chloroform:methanol (99:1 V/V; Randerath, 1966). The chromatography tank was lined with filter paper and the solvents added on the same day that the chromatograms were to be developed. Authentic progesterone and 20 $\alpha$ -hydroxypregn-4-en-3-one were chromatographed adjacent to the samples to aid in the visual detection of the sample steroids under ultraviolet light. The R<sub>p</sub> values (distance of steroid from the origin relative to the position of progesterone) for several authentic steroids using this system are presented in Table 1. The sample steroid and appropriate blank areas were removed from the plates by vacuum and eluted with 10 ml of absolute methanol. Progesterone and 20 $\alpha$ -hydroxypregn-4-en-3-one were quantified by measuring sample absorption at 230 and 240 m $\mu$  in a DU spectrophotometer (Reineke, 1956).

Data on total ovarian weight, progesterone content and concentration and 20 $\alpha$ -hydroxypregn-4-en-3-one content and concentration were analyzed statistically by analysis of variance. (Snedecor and Cochran, 1967).

Table 1. Thin Layer Chromatography of Steroids Using Chloroform:  
Methanol 99:1

	R <sub>p</sub> Value*
Progesterone	1.00
Androst-4-ene-3, 17-dione	.88
3 β-hydroxypregn-5-en-20-one (Pregnenolone)**	.68
20 α-hydroxypregn-4-en-3-one	.55
20 β-hydroxypregn-4-en-3-one	.51
17 α-hydroxyprogesterone	.49
Testosterone	.45

\* 
$$R_p \text{ Value} = \frac{\text{distance of steroid from origin (cm)}}{\text{distance of progesterone from origin (cm)}}$$

\*\* Detected by placing developed chromatogram in an atmosphere of I<sub>2</sub> vapor.

## RESULTS

Experimental data on postpartum ovarian characteristics found in rabbits during this trial are presented in Table 2. Ovaries from suckled rabbits were heavier than ovaries from nonsuckled or control rabbits ( $P < 0.01$ ). In the only other possible comparison ovaries from nonsuckled rabbits tended to weigh less than ovaries from control rabbits but this difference was not statistically significant. Treatment also failed to affect ovarian content or concentration of progesterone or  $20\alpha$ -hydroxypregn-4-en-3-one. Two rabbits, one in the suckled group and one in the nonsuckled group, had unusually high ovarian content and concentration of  $20\alpha$ -hydroxypregn-4-en-3-one (i. e., approximately three times the average based on values from the other five rabbits in their respective groups). A second analysis deleting these high values was performed and it was indicated by this method that ovaries from does in the suckled group had significantly more  $20\alpha$ -hydroxypregn-4-en-3-one content than those from does in the nonsuckled group although a significant difference in hormone concentration was still not suggested. The fact that, based on the legitimate statistical analysis (i. e., using all values), there was no statistically significant difference between groups in progestogen content or concentration while in the same ovaries there was a significant difference between groups in total ovarian weight seems to indicate

Table 2. Mean Ovarian Characteristics of Postpartum Rabbits

Group	Treatment	Total <sup>a</sup> Ovarian Weight, mg.	Progesterone		20 $\alpha$ -hydroxypregn-4-en-3-one	
			Content $\mu$ g.	Concentration $\mu$ g./gm.	Content $\mu$ g.	Concentration $\mu$ g./gm.
I	Control	455.8	4.5	10.1	17.1	30.0
II	Nonsuckled	390.0	4.0	11.2	16.2	41.9
III	Suckled	645.5	5.8	8.8	31.4	47.4
Error mean square <sup>b</sup>		13,980	9.	29.	206	555

<sup>a</sup> Suckled,  $P < 0.01$

<sup>b</sup> Degrees of freedom = 15 for each error mean square

that the increase in gonadal size was not due to ovarian accumulation of these hormones either singly or in combination. Speculation on what did produce the observed effect is presented in the discussion section of this paper.

Rabbits are capable of spontaneous ovulation even though as a rule they are induced ovulators (Walton and Hammond, 1929; Hill, Parkes, and White, 1934; Nalbandov, 1958). In the present study two out of six does in the suckled group possessed functional corpora lutea at autopsy (these structures were enucleated from the stromal tissue before determination of total ovarian weight). In one case the average luteal weight was 6.1 mg. while in the other it was 15.3 mg. If one assumes that the glandular growth rate was comparable in both does these weights suggest that the ovulations occurred at substantially different intervals postpartum. No new ovulations were present at autopsy in the ovaries of rabbits in either the control or the nonsuckled groups.

## DISCUSSION

The results of this experiment indicate that suckling by the litter for 12 days causes a significant increase in maternal ovarian weight in the postpartum rabbit. The nature of the mechanism responsible for this weight increase is not indicated by data collected during the course of this study since no correlations were found between the other ovarian characteristics measured and ovarian weight. More specifically, the weight increase could not be ascribed to an increase in ovarian content of progestogens since there were no significant differences between groups in progesterone and  $20\alpha$ -hydroxypregn-4-en-3-one contents whether these hormones were analyzed singly or in combination. Therefore the observed weight increase in ovaries from rabbits in the suckled group must be assumed to have been caused by some ovarian characteristic or combination of characteristics which were not measured. It is known that the ovarian interstitial tissue in the rabbit functions as a source of  $20\alpha$ -hydroxypregn-4-en-3-one which in turn acts as a positive feedback agent on the anterior pituitary of the mated rabbit to prolong and increase LH discharge (Hilliard, Penardi and Sawyer, 1967; Keys and Nalbandov, 1968). On the other hand, LH stimulates the synthesis and release of this same progestogen from the ovarian interstitial tissue (Hilliard, et al., 1963; Hayward, Hilliard and Sawyer 1964; Spies, Hilliard and Sawyer, 1968). Hilliard

et al. (1968) have presented evidence that indicates that this synthesis and release of  $20\alpha$ -hydroxypregn-4-en-3-one occurs with a concomitant loss of cholesterol stores from the interstitial tissue. In the same study these investigators also reported that chronic administration of prolactin acts specifically on the rabbit ovary to maintain the steroid producing capacity of the interstitial tissue by promoting cholesterol storage. This readily available supply of cholesterol, which of course, serves as a precursor of progestogen synthesis (White, Handler and Smith, 1968) enhances the ability of the ovary to respond to acute release of LH with increased levels of  $20\alpha$ -hydroxypregn-4-en-3-one production. It is proposed that the observed increase in ovarian weight exhibited by suckled does in this study was a result of the influence of the chronic release of endogenous prolactin resulting from nursing of the young by these does. In short it is hypothesized that the observed increase in ovarian weight was due to a greater amount of cholesterol being present in the interstitial tissue of ovaries of suckled does than in the ovaries of does in the control or nonsuckled groups. This hypothesis is in accord with the above cited experimental evidence but conclusive proof of what the causative factor actually is will require direct measurement of cholesterol levels in an experiment of similar design with resultant establishment of positive correlations between cholesterol content and suckling-induced ovarian hypertrophy. Only after this is accomplished will one be able to say with reasonable

confidence that the proposed hypothesis is correct.

One additional observation of interest was the fact that during the course of this study two does in the suckled group ovulated spontaneously. A rough estimation of the time of ovulation based on comparison of average corpora lutea weight with average weight of corpora lutea of known age seemed to indicate that both does ovulated after parturition but at quite different intervals postpartum. The fact that these ovulations both occurred in suckled does would seem to indicate that endogenous levels of plasma LH might have been higher in these ovulating animals but whether this was actually the case is purely conjectural. In fact, Harned and Casida (1969) found a higher proportion of ovulation in nonsuckled primiparous does bred one or four days postpartum than in suckled primiparous does bred at similar intervals postpartum. In addition, hamster LH levels are known to be depressed during lactation (Greenwald et al., 1967). Haun and Sawyer (1961) reported that in the rabbit destruction of areas of the hypothalamus which are known to be necessary for LH release result in increased release of prolactin and consequently they postulated that lactogenesis in the rabbit seems to be a phenomenon in which the adenohypophysis liberates prolactin as a consequence of suppression of the release of LH. This viewpoint is, of course, directly opposed to the suggestion in this study that plasma LH levels might be higher in the suckled rabbits since both cases of spontaneous ovulation occurred in this group.

There is a possibility that the spontaneous ovulations occurred in the same group by chance but this seems to be rather improbable. It would be interesting to pursue this question further but a more productive line of inquiry would probably be to investigate the effects of suckling upon in vivo production of progestogens since this study has indicated that the ovaries of suckled rabbits may possess a greater potential for steroid synthesis as a result of lactation. Preliminary confirmation of the hypothesis concerning increased cholesterol content of ovaries during lactation would be required before embarking on such a study.

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