EFFECT OF LACTATION ON OVARIAN FUNCTION IN THE RABBIT

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Summary. Ovarian function was studied in thirty-eight does suckling at least four, and usually six to eight, young each. A balanced pellet diet was available at all times. Under these conditions, ripe follicles were present in the ovaries but the corpora lutea regressed rapidly following parturition and the size of the ovaries and uterus decreased as suckling continued. A basal progesterone output could not be quantified but the release of 20α -hydroxypregn-4-en-3-one, expressed as $\mu\text{g/g ovary/hr}$, tended to rise throughout lactation. In six of the seven suckled does which were allowed to mate *post partum*, pregnancy was maintained, indicating that sufficient oestrogen was produced by the follicles to maintain luteal function. The results support the concept that neither lactation nor the suckling stimulus suppresses FSH and LH release, since the presumed oestrogen production continues when adequate nourishment is available.

INTRODUCTION

Although there is considerable evidence that ovarian function is affected by lactation and the suckling stimulus (Young, 1961; Perry & Rowlands, 1962), this phenomenon has been little studied in the rabbit. In 1925, Hammond & Marshall reported that if three or more young were suckled, anoestrus set in by Day 12, the ovarian weight was reduced by half mainly because of a decrease in the size and number of the follicles, and the uterus became atrophic; when mating occurred *post partum*, implantation usually resulted but most embryos had resorbed by Day 12. Adams (1967) showed that, if an abundant supply of food was given, pregnancies could be carried to term even in the presence of a large suckling litter, whereas, if little food was given, treatment with pituitary extract maintained pregnancy. These results suggested that regression of the corpora lutea (CL) from *post-partum* mating was due to lack of FSH secretion and follicular development.

Changes in gonadotrophin levels associated with lactation appear to vary with the species of animal studied. In the hamster, no vesicular follicles are present, but elevated LH and depressed FSH secretion probably takes place (Greenwald, 1965; Greenwald, Keever & Grady, 1967); in the mouse, LH

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secretion is preferentially depressed, but a depression in FSH secretion may also occur (Sadler & Browning, 1961); in the rat, follicular quiescence is associated with low FSH and LH release (Rothchild, 1960; Minaguchi & Meites, 1967). Delayed implantation in the last two species is attributed to lack of gonadotrophin, and thus oestrogen secretion, since the administration of either at the appropriate time will permit implantation at the normal time (Weichert, 1942; Whitten, 1955). Output of progesterone and 20α -hydroxypregn-4-en-3-one (20α -OH) from the rat ovary during lactation has been studied by Tomogane, Ota & Yokoyama (1969) who found that progesterone rose from Days 1 to 8 and then decreased, whereas 20α -OH output was high at the beginning and end of lactation but depressed during the intermediate period. Since the doe differs from the rat by not ovulating spontaneously *post partum*, her ovary has no new CL and is in a different physiological state. The rabbit seemed, therefore, an appropriate species in which to study the effects of lactation on ovarian function in the absence and in the presence of induced *post-partum* ovulation. Particularly pertinent to this study was the investigation of ovarian interstitial tissue (IST) activity, since, in the rabbit, this tissue secretes 20α -OH in response to LH stimulation and prolactin has been shown to cause hypertrophy of the interstitial cells and to promote IST cholesterol storage in the hypophysectomized animal (Hilliard, Spies, Lucas & Sawyer, 1968).

MATERIALS AND METHODS

Thirty-eight New Zealand White rabbits were included in this study, all of which were suckling at least four, and usually six to eight, young. Rabbit pellets and water were available at all times and fresh greens were supplied once or twice weekly. The pellets (Ace Hi, California Milling Corp.) contained 18% crude protein, 3% fat, supplements of vitamin B₁₂, D-activated plant sterol, ascorbic acid and minerals.

Progestin assay

Ovarian venous blood was collected under pentobarbital anaesthesia and progestin concentrations were measured as described by Hilliard, Penardi & Sawyer (1967). By this method, blood is collected for 20-min periods and progesterone and 20α -hydroxypregn-4-en-3-one (20α -OH) are measured spectrophotometrically following paper chromatography. Four μ g or more of each compound are quantifiable; thus, when expressed on a μ g/hr basis, quantities of 12 μ g or more are necessary for accurate quantification.

The luteinizing hormone used was NIH-LH-s15, which had a potency of 0.99 times the NIH-LH-s1 standard.

RESULTS

Effect of lactation on the ovary and uterus

Table 1 shows that, following parturition, there is a marked drop in ovarian weight which remains low for at least 5 weeks if suckling continues. No consistent decrease in follicular size was evident and vesicular follicles 1 mm or

more in diameter were always present. The CL of pregnancy regressed rapidly within the first 72 hr after parturition, and by Days 4 to 5, had become white, compact corpora albicantia weighing no more than 6 mg each. This rapid luteal involution was no doubt largely responsible for the reduction in ovarian weight.

Ovulation was induced in three animals given a minimal ovulating dose of LH (2 µg/kg i.v.) on Day 6 or 10 of lactation, indicating that functional follicles

TABLE 1
EFFECT OF LACTATION ON THE OVARY AND UTERUS

Day of lactation	Wt of young (g)	Ovary wt (mg)	CL wt (mg)	Approx. max. foll. size (mm)	Uterus diam. (mm)	Rabbits ovulating to LH†
1 to 2 (5)*	—	655±93	13.3±1.3	1.7±0.8	21.2±0.4	1/1 2/2
4 to 9 (8)	126±16‡	406±41	4.7±0.4	1.5±0.2	10.5±0.1	
10 (5)	138±7	441±117	<5	1.7±0.2	8.2±0.9	
19 to 24 (7)	300±26	347±11	<5	1.6±0.2	6.2±0.5	
29 to 34 (3)	643±250	450±67	<5	≤2.0	—	

* No. of animals shown in parentheses.

† Dose given was 2 µg/kg NIH-LH-s15 i.v.; potency 0.99 times NIH-LH-s1.

‡ Mean±S.E.

were being maintained by endogenous gonadotrophin. In spite of this, the uteri tended to become pale, flaccid and reduced in size, showing evidence of oestrogen deprivation.

Progestin release during lactation

Table 2 shows the basal and stimulated outputs of progesterone and 20 α-OH as measured in the ovarian venous effluent before and immediately following an i.v. injection of 2 µg/kg LH. The basal levels of progesterone were too low for quantification by our procedure, but basal as well as stimulated levels of 20α-OH tended to increase from Day 4 of lactation. Some progesterone secretion could also be detected following LH stimulation in ten of the twelve suckled does cannulated between Days 10 and 24 *post partum*. Since only corpora albicantia, weighing approximately 3 mg each, remained at this time, the ovarian ist was the probable source of both steroids. Levels of basal and stimulated 20α-OH secretion during lactation were generally higher than those secreted in oestrous rabbits (Hilliard, Archibald & Sawyer, 1963) and were similar to those recorded at the end of pregnancy (Hilliard, Spies & Sawyer, 1968).

Concurrent lactation and pregnancy

Although spontaneous *post-partum* ovulation does not occur in rabbits, does are in oestrus during the first 48 hr and will copulate and ovulate readily. Table 3 shows that animals, which were allowed to mate *post partum*, maintained higher ovarian weights which were similar to those observed in non-lactating pregnancies (Hilliard, Spies & Sawyer, 1968). In three does mated

TABLE 2
PROGESTIN RELEASE FROM THE OVARY DURING LACTATION

Day of lactation	Control output			Stimulated† output		
	Progesterone (µg/g ovary/hr)	20α-OH (µg/g ovary/hr)	No. of animals in which progesterone was detectable	Progesterone (µg/g ovary/hr)	20α-OH (µg/g ovary/hr)	No. of animals in which progesterone was detectable
1 to 2 (5)*	0	66 ± 10†	1/5	< 12	675 ± 46	2/4
4 to 9 (6)	0	189 ± 95	0/6	17 ± 15	862 ± 431	2/4
10 to 12 (5)	< 12	233 ± 90	2/5	97 ± 29	1098 ± 118	5/5
19 to 24 (7)	0	130 ± 47	0/7	73 ± 23	868 ± 224	5/7

* No. of animals shown in parentheses.

† At the end of a 20-min control blood collection, 2 µg/kg of NIH-LH-s15 was injected i.v., and blood for the stimulated output was collected during the succeeding 20-min period. Potency of the LH-s15 is 0.99 times NIH-LH-s1.

‡ Mean ± S.E.

TABLE 3
EFFECT OF LACTATION ON OVARY AND PROGESTIN SECRETION IN DOES MATED WITHIN 48 HR POST PARTUM

Day of lactation	No. young suckled	Ovary wt (mg)	CL wt (mg)	Follicle size (mm)	Rabbits ovulating to LH†	Viable foetuses at Days 10 to 12	Progesterin output (µg/g ovary/hr)		
							Control	Stimulated‡	
5 to 6 (3)* 10 to 12 (5) 30 to 32 (2)	4 to 8 8 4, 7	738 ± 3† 726 ± 64 Delivered living young at term	7.8 ± 0.9 11.8 ± 2.1	1.2 ± 0.2 1.4 ± 0.2	1/3 3/3	— 4/5 2/2	Prog.	20α-OH	20α-OH
							12 ± 9 41 ± 21	112 ± 23 140 ± 23	320 ± 88 1435 ± 190
							Not cannulated		

* No. of animals shown in parentheses.

† Dose used was 2 µg/kg LH-s15 i.v.

‡ Mean ± S.E.

post partum which received 2 µg/kg LH i.v. on the 6th day of lactation, there was no evidence of implants and the CL induced by the *post-partum* mating had regressed by Days 10 to 12, although only one of the three had ovulated in response to the exogenous LH. On the other hand, four of five animals which were each nursing eight young had functional CL and viable foetuses. In these animals, the basal and stimulated levels of progesterone did not differ significantly from those of pregnant, non-lactating rabbits, but the stimulated levels of 20 α-OH at Days 10 to 12 appeared to be somewhat elevated. Two animals which were not cannulated or given exogenous LH following *post-partum* mating suckled four and seven young respectively and delivered living litters at term.

DISCUSSION

A progressive decrease in ovarian weight and uterine size and tonicity was apparent in the present series of suckled does during the first 24 days *post partum*, thus confirming the observations of Hammond & Marshall (1925). In our animals, however, the reduction in ovarian weight appeared to be due mainly to the rapid regression of the CL of pregnancy rather than to a marked change in follicle size and, in the three animals tested on Day 6 or 10 of lactation, ovarian follicles responded by ovulating to a minimal ovulating dose of LH. The small size and flaccid appearance of the uteri gave evidence of oestrogen deprivation, although follicles could be ovulated by administering LH. Since oestrogen production by the follicles is clearly essential to sustain luteal development and function in the rabbit (Keyes & Nalbandov, 1967), luteal weight, progesterone secretion and viable foetuses all indicated that oestrogen was being secreted from the follicles in amounts compatible with the maintenance of pregnancy. On the other hand, a single injection of LH, within 4 or 5 days of *post-partum* mating, resulted in luteal regression, presumably by luteinizing the follicles and thus removing the source of oestrogen.

High levels of 20α-OH secretion following LH stimulation indicated that sterol precursors were being stored by the ovarian IST during lactation, as in intact and hypophysectomized rabbits treated with prolactin (Hilliard, Spies, Lucas & Sawyer, 1968). The release of progesterone after LH stimulation showed that healthy CL are not a prerequisite for the synthesis and release of this steroid, and implied that progesterone is synthesized by the interstitium and that its release, as well as that of 20α-OH, is stimulated by LH.

Prolactin secretion has been associated with evidence of FSH and LH suppression under a wide variety of conditions which alter hypothalamo-pituitary connections or affect the central nervous system. These include median eminence lesions, pituitary stalk section, treatment with tranquillizing drugs, and a variety of non-specific stimuli such as heat, cold, electric shock, and physical and psychic trauma (Rothchild, 1965). Whereas it is possible that the suckling stimulus might act similarly to suppress FSH and LH discharge, the depressed ovarian function observed in suckled does by Hammond & Marshall (1925) seems more directly related to lack of food, since in our animals, as well as in those studied by Adams (1967), ample food intake permitted concurrent lactation and pregnancy.

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REFERENCES

- ADAMS, C. E. (1967) Concurrent lactation and pregnancy in the rabbit. *J. Reprod. Fert.* **14**, 351.
- GREENWALD, G. S. (1965) Histological transformation of the ovary of the lactating hamster. *Endocrinology*, **77**, 641.
- GREENWALD, G. S., KEEVER, J. E. & GRADY, K. L. (1967) Ovarian morphology and pituitary FSH and LH concentration in the pregnant and lactating hamster. *Endocrinology*, **80**, 851.
- HAMMOND, J. & MARSHALL, F. H. A. (1925) *Reproduction in the rabbit*, p. 122. Oliver & Boyd, Edinburgh.
- HILLIARD, J., ARCHIBALD, D. & SAWYER, C. H. (1963) Gonadotrophic activation of preovulatory synthesis and release of progesterin in the rabbit. *Endocrinology*, **72**, 59.
- HILLIARD, J., PENARDI, R. & SAWYER, C. H. (1967) A functional role for 20 α -hydroxypregn-4-en-3-one in the rabbit. *Endocrinology*, **80**, 901.
- HILLIARD, J., SPIES, H. G., LUCAS, L. & SAWYER, C. H. (1968) Effect of prolactin on progesterin release and cholesterol storage by rabbit ovarian interstitium. *Endocrinology*, **82**, 122.
- HILLIARD, J., SPIES, H. G. & SAWYER, C. H. (1968) Cholesterol storage and progesterin secretion during pregnancy and pseudopregnancy in the rabbit. *Endocrinology*, **82**, 157.
- KEYES, P. L. & NALBANDOV, A. V. (1967) Maintenance and function of corpora lutea in rabbits depend on estrogen. *Endocrinology*, **80**, 938.
- MINAGUCHI, H. & MEITES, J. (1967) Effects of suckling on hypothalamic LH-releasing factor and prolactin inhibiting factor, and on pituitary LH and prolactin. *Endocrinology*, **80**, 603.
- PERRY, J. S. & ROWLANDS, I. W. (1962) *The ovarian cycle in vertebrates*. In: *The Ovary*, Vol. 1, p. 275. Ed. S. Zuckerman. Academic Press, London.
- ROTHCHILD, I. (1960) The corpus luteum-pituitary relationship: the association between the cause of luteotrophin secretion and the cause of follicular quiescence during lactation; the basis for a tentative theory of the CL-pituitary relationship in the rat. *Endocrinology*, **67**, 9.
- ROTHCHILD, I. (1965) Interrelations between progesterone and the ovary, pituitary, and central nervous system in the control of ovulation and the regulation of progesterone secretion. *Vitam. Horm.* **23**, 209.
- SADLER, W. A. & BROWNING, H. C. (1961) Gonadotrophic secretion in lactating mice. *Proc. Soc. exp. Biol. Med.* **106**, 558.
- TOMOGANE, H., OTA, K. & YOKOYAMA, A. (1969) Progesterone and 20 α -OH pregn-4-en-3-one levels in ovarian vein blood of the rat throughout lactation. *J. Endocr.* **44**, 101.
- WEICHERT, C. K. (1942) The experimental control of prolonged pregnancy in the lactating rat by means of estrogen. *Anat. Rec.* **83**, 1.
- WHITTEN, W. K. (1955) Endocrine studies on delayed implantation in lactating mice. *J. Endocr.* **13**, 1.
- YOUNG, W. C. (1961) *The mammalian ovary*. In: *Sex and Internal Secretions*, Vol. 1, 3rd edn, p. 449. Ed. W. C. Young. Williams & Wilkins, Baltimore.