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The nature of the amenorrhea of lactation in humans.

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THE NATURE of the AMENORRHEA of LACTATION in HUMANS

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THE NATURE of the AMENORRHEA of LACTATION in HUMANS

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CONTENTS

I INTRODUCTION

The idea, popular among the laity, that the amenorrhea associated with the period of lactation gave protection against conception is, of course, known to the medical profession to be unfounded. It is obvious to those interested, however, that not all women fail to menstruate during lactation and that pregnancy does occur in a reasonable number of instances either before menses resume or after the cycle has re-established. Likewise, the reappearance of menses does not seem to decrease or influence the secretion of breast milk, therefore, the endocrine systems responsible for lactation and menstruation may not necessarily be reciprocally related.

An observation made some years ago and reported in 1943 by Fortune indicates that Arapesh women do not resume menses during lactation before the infant has cut its first incisors, although Caucasian women may menstruate earlier. These women are an inbred group (8000 or less) with a characteristic long lactogenic interval. Nursing for three years, which is common among these women, does not produce amenorrhea for that length of time. (1).

With these simple and gross observations in mind it was decided to study the problem of the Nature of the Amenorrhea of Lactation in Humans.

II REVIEW OF LITERATURE

A careful review showed thearticles written on this subject to be sparsely distributed throughout the literature.

Everyone agreed about the growth and development of the breast and the process by which lactation occurs, but the effect of lactation on the menstrual cycle has not been well described.

Novak and Hoskins agree on the theory that at puberty the ovarian hormones, estrogen and progesterone, will stimulate the anterior pituitary to increase the secretion of a duct growth factor (mammogenic hormone). During pregnancy progesterone plus estrogen (placental hormones) causes an increased secretion of lobule-alveolar growth factor and is responsible for proliferation of alveolar ducts and lobules. Theories about why lactation does not occur during pregnancy are, 1. that the placenta produces a lactation suppressing agent, 2. that the distended uterus inhibits lactation, 3. that the secretion of progesterone by the corpus luteum during pregnancy inhibits lactation, 4. that the lactogenic hormone is inadequate during pregnancy and is increased 2-4 times within a short time after parturition. Novak's only comment about menstruation and ovulation during lactation was to reiterate that ovulation is followed by menstruation if fertilization does not take place, therefore, menstruation without ovulation is possible but ovulation without menstruation is impossible unless pregnancy intervenes.

Hoskins (2) assumes that menstrual failure during lactation is due to the action of a mammary hormone exerted either

directly on the ovaries or via the anterior-pituitary to cause a persistence of corpus luteum which in turn arrests the cyclic process. It is more probable that the Prolactin Hormone is responsible for this.

One of the most informative papers published was one by Paul Topkins (3) in 1943. His purpose was to determine the extent of ovarian inhibition during lactation. For his study he chose to examine the endometrium by biopsy during the period of lactation. He assumes that if progestational (progesteronal) endometrium is formed, then ovulation has taken place and that the absence of such an endometrium indicates, but does not prove, an absence of ovulation, (4). He further states that failure of ovulation cannot definitely be established without microscopic study of the ovaries unless you assume ovulation is always followed by luteinization and that luteinization always produces a secretory endometrium.

Since the endometrium usually regenerates in three weeks post-partum, except at the placental site, where about seven weeks is required, Topkins began his biopsies at the 6th post-partum week. He took 145 specimens from 28-women, eight colored and twenty white, who had had previous regular periods before pregnancy and who were otherwise gynecologically normal. Four biopsies were taken from each patient and classified as estrogenic and progestational and the number of weeks post-partum noted. One hundred thirty six (136) or 94% proved to be estrogenic - and of that 94% - 15% were poorly developed or hypo-

estrogenic whereas 85% were fairly well developed and constant throughout the amenorrhea, showing mid-follicular phase (end of 1st week of cycle) endometrium. The remaining 6% showed progestational endometrium and all were associated with the onset of the first menstrual flow.

Topkins concluded that (1) the endometrium in lactation amenorrhea shows diminished estrogen stimulation, (2) during lactation the ovarian cycle is suppressed completely in amenorrhea and incompletely in lactation menstruation, (3) on the basis of animal experimentation, inhibition of ovarian activity is the result of suppression of gonad atrophic activity of the hypophysis and that (4) suppression of gonadatrophic activity, in turn, is due to the action of prolactin or else to a hormone of the lactating mammary gland not yet isolated.

He suggests that 85% of cases of undifferentiated endometrium, as in the first week of the cycle, indicates that the follicle is not fully developed and that 15% of cases with hypoplasia suggest that those follicles are regressing. This study indicates that in lactation amenorrhea there is inhibition of ovarian activity, the follicles failing to mature to the point of rupture. Topkins also further concludes that it is reasonable to assume if luteinizing hormone is responsible for lactation amenorrhea then ovulation would be present, which it is not.

We now know, however, that pure Follicle Stimulating Hormone will stimulate the ovarian follicle to the point of morphological development but does not cause the secretion of estrogen.

The addition of Luteinizing Hormone (L.H.) is necessary to produce estrogen and for rupture of follicles to occur, (5).

Another careful study is reported by Cherry (6), who in 1986-37 described the action of the mammary gland on the ovary. His paper states that the mammary gland develops under the influence of estrogen and when primed by the corpus luteum hormone the administration of estrogen will stimulate lobular-alveolar development. During pregnancy estrogen is present in large amounts in the circulation, reaching a peak shortly before parturition and falling markedly after parturition. This fall in estrogen releases the inhibitory action on the pituitary gland and prolactin or lactogenic hormone is released under the sucking reflex stimulus, which is a function of the hypothalmus. Cherry then explains the lack of menstruation in a majority of women during lactation on the basis that there is a low estrogen level in the circulation that is inhibited by some antagonistic hormone action from the lactating breast, preventing the maturation of the ovarian follicle. This action, he thinks, varies in intensity and it is assumed that when ovulation , menstruation or pregnancy do occur during lactation that the pituitary has overcome this inhibition. Cherry, too, assumes the ovarian failure is probably secondary to pituitary suppression. Cherry's investigation was based on a theory that the breast produced a substance at the beginning of lactation which inhibited maturation of the ovarian follicle and ovulation. He injected alcohol precipitates of pregnancy urine and extracts of lactating mammary

gland into albino rats. In the gonadatrophic injected controls corpora hemorrhagica and corpora lutea developed in every instance. In the controls injected with extracts of mammary gland no change was noted either macroscopically or microscopically. When the two extracts were mixed in the same syringe and given together the result in most instances was complete absence of corpora hemorrhagica and corpora lutea. Follicular development was present in the majority of instances, but in some animals this too was absent, as confirmed by negative vaginal smears and microscopic sections.

Further work on the rabbit proved there was a definite inhibitory action of the lactating mammary gland on the ovary.

Griffith and McBride (7) in 1939 reported 21-post partum patients examined and biopsied at intervals varying from three to twenty-four weeks. Ten patients nursed their babies from two to nine months and eight of these, 80%, began to have menstrual periods and ovulation only after weaning. The average duration of nursing was 3½-months, followed by anovulatory flow at five months and by the first ovulation at 5½-months. Two patients, 20%, had regular menses (ovulatory) during their period of lactation. One of these had menstrual periods beginning after two months, ovulation after five months. She nursed her child seven months. Another had menstrual periods re-established after six months, ovulation after 6½-months. She weaned her child at age nine months.

Eleven patients unable to nurse babies had menstrual periods beginning on an average of two months post-partum and ovulation at five months post-partum or later.

In 95% of these cases an anovulatory flow preceded ovulation. These authors assume, as does Topkins, that Pituitary Gonadatrophin is immediately suppressed, probably due to the lactogenic hormone or some other unknown internal secretion.

Further studies along the same theme done by Kurzrok, Lass and Smelser (8) consisted of biopsies done on thirty women who were simultaneously lactating and having regular menstrual cycles. They establish in their series that 65% of these cycles are sterile or anovulatory periods. A year later(9), in 1938, these same authors presented another study relating to the time of human ovulation during lactation, in which they again biopsied post-partum women who were nursing regularly and having regular catamenia. The presence of secretory endometrium indicates ovulation and the presence of non-secretory endometrium within ten days of the succeeding menstrual flow indicates an anovulatory cycle in their series. Forty-seven women were studied by biopsy at four week intervals and 55% of these cycles were anovulatory and 45% were ovulatory.

Pregnandiol output in the urine is now accepted as an index of corpus luteum activity in both the pregnant and non-pregnant woman. Fifty-one cases of pregnandiol excretion were studied during the first post-partum week by Wilson, Randall and Osterbery showing evidence that some pregnandiol is present immediately after parturition but in amounts less than those found ante-partum (10). Estimates of pregnandiol on the 4th post-partum day showed 45% negative, 21% minimal and 34% with a mean value of 2.75 to 6-mgms per 24-hours. This may indicate some corpus

luteum function post-partum and its association with lactation is not yet known.

Robinson (11) discusses the subject from the opposite point of view, namely: if menstrual periods are resumed or if pregnancy does occur during lactation does milk secretion stop? He interviewed over 600 mothers regarding the age of weaning and the time their menstrual periods resumed. He concluded that menstruation is neither a reason for weaning nor a cause for failure of lactation; these functions seem to be independent of lactation. In the series of women pregnant and simultaneously lactating, many pregnancies went unnoticed until foetal movements were felt. By this time the foetus is 18-20 weeks old and the nursing infant seven months old which means that adequate lactation had continued for five months of pregnancy. By seven months lactation normally diminishes so the lowered output after that could not be attributed to the pregnancy.

Other work based on animal experimentation has been published and where possible has been applied in theory to humans. Bates, Lahr and Riddle (12) in 1935 showed that data concerning the action of F.S.H. and prolactin on the ovary and sex accessories of fowls fell into two contrasting groups demonstrating directly opposite effects. Prolactin stopped ova production and caused decrease in ovarian weight. F.S.H. stimulated ova production, stopped laying and increased ovarian weight markedly. Prolactin decreased oviductal and uterine size, comb size and pubic bone width, whereas F.S.H. increased the size of these structures.

Lahr and Riddle (12) injected Prolactin into non-lactating rats and observed suspension of two to four estrous cycles with large undegenerated corpora lutea in the ovaries. Progesterone produced no such effect. Riddle and Bates (13) in 1933 showed prolactin to cause regression in the weight of the testis of a pigeon and concluded the same to be true of the ovary. They therefore offer the possibility that the gonad-stimulating (sex-maturing) hormone is held in abeyance by circulating prolactin. Turner and Meitus (14-15) showed that in rabbits the lactogenic hormone which was low during pregnancy increased 2 - 4 times after parturition and this increase was not influenced by a simultaneous pregnancy. Finally in 1934 Collip, Selye and Thomson (16) presented the idea that the stimulus of sucking was also involved in this problem via the hypothalmus. They believe suckling stimulates the anterior-pituitary to produce prolactin and to modify its activity toward the ovary so that corpora lutea of post-partum ovulation long retain their healthy structure and active function while further ovulation and phenomena of estrus are inhibited.

From these articles it is evident that much is still to be learned about the physiological actions of these endocrine glands during lactation.

Further light was shed on the subject by Albright, Smith and Dodge (17) when in their presentation of a new method of assay of pituitary F.S.H. they review a case of secondary amenorrhea with persistent lactation after 2½-years. The F.S.H. output per-24-hours in this patient fell within the normal

limits instead of being suppressed and they promised a further investigation of the problem of lactation amenorrhea.

III PURPOSE

The idea that the F.S.H. output per 24-hours may consistently be within normal limits is the basis for the following investigation. The hormone level will be determined by urinary assay and correlated with the simultaneous endometrial biopsy findings in an attempt to not only confirm other work done on the incidence of ovulation during lactation but also to determine the true status of the pituitary activity.

IV EXPERIMENTAL PROCEDURE

Thirteen colored females were selected from the Post-Partum Clinic at the Louisville General Hospital at the time of their regular six weeks post-partum examination. These women were biopsied and an assay of urinary F.S.H. was taken simultaneously. All of the patients were in good general health and had had no previous menstrual irregularities. These women were examined at various intervals between their 6th and 19th post-partum week. The incidence of non-secretory and secretory endometrium was observed; the level of Pituitary F.S.H. output per 24-hours was determined and the presence or absence of menstruation was noted during their period of lactation . The data is compiled in charts and is here discussed. One case

of persistent amenorrhea in a patient 14-months post-partum is presented.

Discussion regarding the method of hormone assay is needed. The procedure used is that described and confirmed by Smith, Albright and Dodge, (17-18), in 1943. An eight hour overnight urine specimen is filtered and if necessary acidified with glacial acetic acid. One gram of sodium chloride per 100 cc of urine is added and four volumes of 95% ethyl alcohol. This is cooled overnight in the refrigerator and the precipitate collected in a 250 cc centrifuge bottle. The precipitate is washed with anhydrous ether and dried overnight in a vacuum dessicator. The precipitate is then redissolved in water, 30-45 cc, and dialysized for four hours against running tap water to remove impurities and render the specimen less toxic. The product of dialysis is re-precipitated by adding .1 gm NaCl to the whole specimen (45-cc) and four volumes of ethyl alcohol. This is cooled overnight and the precipitate collected in a 50 cc centrifuge tube and again dried with anhydrous ether overnight in a dessicator. This precipitate is stable at room temperature and when ready for use is dissolved in 5.5 cc of distilled water and kept refrigerated until the assay is completed. White mice 19 to 20 days old and weighing 7-10 gms are used for the assay and are injected subcutaneously in the back with 2½-cc of test solution over a period of three days, receiving ½ cc at each injection. Twanty-four hours after the last injection the mouse is autopsied and the uterine weight determined by removing it, pressing it between filter paper to remove the excess tissue fluid and weighing it on a balance.

One mouse unit of hormone is the amount required to produce 100-150% increase in the size of the uterus (weight). Normally the uterus in animals injected with saline as controls weighed 3.7 mgs ∕ or - .9 mgms as reported by Albright, et al. In this series, and with this particular strain of mice, 14 animals kept as controls had an average uterine weight of 3.28 mgm. The final precipitate contains all the hormone excreted by that patient in an eight hour period, therefore if 2.5 cc of this 5.5 cc solution contains one or more Mouse Units (Mu) as indicated by a positive test, then the 5.5 cc will contain 2.2 Mu. The patient's output for 24-hours would then be 6.6 Mu. By making appropriate dilutions with water the original specimen may be further assayed for higher levels of F.S.H.

The level for normal regularly menstruating women, not pregnant and not lactating, is more than 6.6 and less than 53 Mu units in 24-hours, according to standards reported by Albright, Smith and Dodge. These authors stress the importance of the F.S.H. test, in that at present it is the only anterior pituitary hormone that can be assayed easily and also suggest that it be applied further clinically. The procedure is simple in technique, its main fault being the amount of time required to prepare one specimen for assay (44-92 hours) - (5).

Regarding the use of the alcohol precipitate test, Heller and Heller (19) cite five advantages of this method over other extractions, chiefly the tannic acid or tungstic acid precipitates. It is simple and direct, the products are stable for storage at any stage, the F.S.H. is concentrated many times,

dialysis removes a large percentage of the toxicity (probably by removal of salts), potency is not affected by detoxification, just as large or larger amounts of hormone are recovered with this method. The use of the mouse uterus as the endpoint for the assay was established by Levin and Tyndale (20). This proved to be a more accurate and more sensitive test than did follicular enlargement, increase in ovarian weight or vaginal canalization. These authors established the absence of estrogenic substances in the urine by injections into spayed rats as well as by the fact that all potency was destroyed by heating, acid treatment and other procedures which would not injure estrin, had it been present. The uterine response was 67% greater if mice were autopsied 72-hours after the first injection.

The endometrial biopsies were made with a Novak curette, after cleansing the cervix with Merthiolate. The tissue obtained was fixed with Formalin (10%), sectioned and stained with H & E. as a routine procedure of this clinic. Classification as to secretory or non-secretory activity is made on the basis of the standard text books of Gynecology (Novak).

A. Acidified Filtered Urine with Alcohol Added.
B. First Precipitate.
C. Dialysis Process.
D. Dialysized Specimen with Alcohol Added.
E. Second Precipitate.
F. NaCl & Balance.

A. Biopsies - sections.
B. Index File.
C. Mouse board - 1cc syringe - Specimen Bottles.
D. Usual type breeding pan.
E. Autopsied mice.

V LACTATION AMENORRHEA DATA

PATIENT	HISTORY	BIOPSY	F.S.H. (Mu)	COMMENT	WKS. POST-PARTUM
I L.A. 24-yr. C.F.	Para iii (3/3/47). Did not menstruate with prev. lact. No preg. during prev. laction.	5/2/47-Int. non-sec. (sl. hyperplasia).	∠6.6 -26 -53 19.8mg. 4.4 mg. 4.4 mg.	Lactating. No menses.	8
		5/16/47-Post. mens. non-sec.	∠6.6 ∠26 ∠53 79 mg. 38.2mg. 51 mg. 33.1mg. 26.3mg.	Lactating. No menses.	10
		5/29/47-Int. non-sec.	∠6.6 -26 -53 29.3 mg. 6.1 mg. 5.5mg.	Lactating No menses.	12
		6/13/47-Int. non-sec.	∠6.6 ∠26 -53 69.2mg. 21mgs. 6.5mg.	Lactating. No menses.	14
		6/30/47-Int. non-sec.	∠6.6 -26 -53	L.M.P. 6/13 - 17/47.	16
2. M.E.B. 24-yr. C.F.	Para iii (3/2/47). L.M.P. 3-days beg. 4/11/47. No cramp. Did not mens. during lactation.	4/11/47-Int. Non-sec. (sl. hyperplasia).	∠6.6 ∠26 -53 32.1mg. 15.2mg. 5.2mg.	Bleeding 3-days beg. 4/11/47.	6
		4/25/47-Int. non-sec.	∠6.6 ∠26 -53 66 mg. 19.1mg. 5.1 mg.	Lactating.	8
		5/16/47-Post-mens. non-sec.	∠6.6 -26 -53 10.7 mg. 3mg.	Scant bleeding 1½-days beg. 5/11/47. Lactating.	11
		6/20/47-Int.non-sec.	∠6.6 -26 12.8 mg. 5mg.	L.M.P. 6/17/47. Scant one day. Lactating.	16

PATIENT	HISTORY	BIOPSY	F.S.H.(Mu)	COMMENT	WKS.POST*PARTUM
3. L.B. 27-yr. C.F.	Para i (2/20/47). Lactating. No menses.	4/4/47-Int. non.sec. Sl. hyperplasia.	∠6.6 -53 8.4 mg. 3.5 mg.	Lactating.	6
		4/18/47-Int.nonsec. (Thrombosis)	-6.6 -6.6 6.4 mg. 3.5mg.	Lactating.	8
		5/2/47-Int.non-sec.	∠6.6 -26 -53 21mg. 4.5mg. 4.3mg.	Lactating. No menses.	10
		5/16/47-Int. non-sec.	∠6.6 -26 -53 49.3mg. 3.3mg. 3.3mg.	Lactating. No menses.	12
4. E.B. 29-yr. C.F.	Para vi (2/6/47). Lact. No menses. Did mens. during prev. lact. Did become pregnant during a previous lactation.	4/11/47-Int. non-sec.	∠6.6 ∠26 -53 40.8mg. 12.2mg. 5.4mg.	Lactating.	9
		4/25/47-Int. non-sec.	∠6.6 -26 -53 24.1mg. 5.2mg. 4.8mg. 4.3 mg.	Lactating. No menses.	11
		5/9/47-Int. non-sec.	-6.6 -6.6 5.1mg. 3.0 mg.	Lactating. No menses.	13
		5/23/47-Int. non-sec.	∠6.6 -26 -53 9.2mg. 3.0mg. 3.2mg.	Lactating. No menses.	15
5. R.C. 16-yr. C.F.	Para i (2/16/47) Lactating. No menses.	4/4/47-Int. non-sec.	∠6.6 ∠26 -53 30.6mg. 20mg. 5.6mg.		7
		4/18/47-Int. non-sec.	∠6.6 ∠26 -53 57.2mg. 10.2mg. 3.9mg.	Lactating. No menses.	9

%%%

PATIENT	HISTORY	BIOPSY	F.S.H.(Mu)	COMMENT	WKS.POST-PARTUM
6. L.B. 21-yr. C.F.	Para ii (1/25/47) Lactating. No mens. No mens. during prev. lactation.	4/25/47-Int. non-sec. Old decidual reaction.	∠6.6 ∠26 -53 27.7mg. 10.5mg. 4.6mg.	Nursed 1st baby 9-mos. Mens. @ 13-mos.	13
		5/9/47-Int. non-sec.	∠6.6 ∠26 -26 -53 33mg. 12mg. 3mg. 3mg. 3mg.	Lactating. No mens.	15
		5/23/47-Int. non-sec.	∠6.6 ∠26 -53 28.4mg. 20.6mg. 4.1mg.	Lactating. No menses.	17
		6/6/47-Int. non-sec.	∠6.6 ∠26 -53 22 mg. 17mg. 4.3mg.	Lactating. No menses.	19
7. L.S. 42-yr. C.F.	Para iv (3/8/47)	4/24/47-Int. Non-sec. (Atrophy of stroma)	∠6.6 -26 -53	Lactating. Irregular spotting.	7
8. N.M. 27-yr. C.F.	Para ii (3/12/47) Lact. No mens. No mens. or preg. dur. prev. lact.	5/9/47-Int. non-sec.	∠6.6 -26 -53 36mg. 3.0mg. 3.0mg. 3.0mg.		8
		5/23/47-Int. non-sec.	∠6.6 ∠26 -53 19.8mg. 9.1mg. 5.2mg	Lact. Exam shows normal mens. flow.	10
		6/27/47-Int. non-sec.	∠6.6 -26 -53	L.M.P. 5/23/47. One day. Lactating. No period in June.	14
9. P.H. 31-yr. C.F.	Para iii (3/6/47) Lact. No mens. No.mens. or preg. during prev.lact.	5/9/47-Int. non-sec.	Specimen Toxic. Mice died.	Pds. resumed 1-mo. after lact. stopped with prev. preg.	9

PATIENT	HISTORY	BIOPSY	F.S.H.(Mu)	COMMENT	WKS.POST-PARTUM
10. E.K. 30-yr. C.F.	Para v (3/5/47) 14-mos. post-part. no mens. Nursed other babies 6-mos. Did not mens. dur. lact. Resumed Catamenia 3-mos after weaning.	5/20/47-Int. non-sec. Atrophy of endometrium. 6/30/47-Int. non-sec.	~~46.6~~ 426 -53 35.3mg. 10.3mg. 3.0 mg. 46.6 426 -53	Lactating. Breasts congested. No menses. Not lactating.	62 68
11. M.D. 21-yr. C.F.	Para ii (4/4/47) Lact. No mens. Did not nurse 1st. baby. Catamenia resumed @ 2½-mos. post-partum.	5/16/47-Int. non-sec.	46.6 -26 -53 36mg. 3.0mg. 3.0mg. 3.1 mg.		6
12. C.G. 18-yr. C.F.	Para i (4/5/47) Lactating. No mens.	5/23/47-Int. non-sec. Thrombosis and hyanilization.	46.6 -26 -53 10.1mg. 4.0mg. 3.6mg.		7
13. M.W. 20-yr. C.F.	Para i (4/10/47) Lact. No mens.	5/23/47-Late Int. Sec. 6/13/47-Int. non-sec. 6/20/47-Int. non-sec. 6/27/47-Int. non-sec.	46.6 -26 -53 35 mg. 3.8mg. 3.6 mg. 46.6 426 -53 20 mg. 12.2mg. 4.1mg. Specimen Toxic Mice died. 46.6 426 -53	(Should mens.within eight days. L.M.P. 1½-days beg. 5/24/47. Abscess of breast rupt. spont. Rx Stilbesterol 5-mg. daily x 5. Not lactating. Abscess healing. L.M.P. 3-days beg. 6/24/47. Scanty.	6 9 10 11

The data in the preceding chart may be tabulated somewhat as follows:

Thirty-four F.S.H. determinations on 13 patients were done and 36-biopsies are reported. Two F.S.H. specimens were toxic and the mice assayed with these samples died before receiving the final injection.

The incidence of non-secretory endometrium was 97% (35 biopsies). The corresponding F.S.H. assays showed; 13 specimens positive for 6.6 Mu and negative for 26 Mu, and 17 specimens positive for 26 Mu but negative for 53 Mu, giving a total of 30 F.S.H. specimens well within the normal limits of daily output for regularly menstruating, non-pregnant, non-lactating women. The incidence of normal F.S.H. in percent in this series is therefore 91%. Two F.S.H. levels (6%) were less than 6.6 Mu per 24-hours and these were associated with a non-secretory endometrium. One F.S.H. level (3%) was above 53 Mu but below 85 Mu per 24-hours and this was associated with a non-secretory endometrium. All three patients showing other than normal F.S.H. levels on one occasion had at least 3 or 4 other assays with the normal levels.

One biopsy of late interval secretory endometrium was obtained, the incidence being 3% (case 13). This was associated with a normal F.S.H. level (more than 6.6 Mu, less than 26 Mu per 24-hours) and the patient had a scanty menstrual period which began about 36-hours after the biopsy was taken.

Four patients (30.7%) had catamenia resumed during this period of observation and of these, three were anovulatory and one ovulatory in nature (case 1,2,8, & 13). Nine cases (69.3%) were complete amenorrheas throughout the period of observation.

One patient with a breast abscess is interesting in that at six weeks post-partum a biopsy of secretory endometrium was obtained, and the patient had a scanty menstrual period beginning 1½-days later. At 9 weeks the patient was seen again, at which time she had a breast abscess which had ruptured spontaneously. The endometrium on that day was non-secretory. The local breast lesion was treated and the patient given 5 mgm. of Stilbesterol daily for 5 days to dry up her breasts, and the baby weaned. At ten weeks post-partum (one month after the secretory biopsy) this patient showed a non-secretory endometrium and four days later had a scanty 3-day menstrual period. At 11 weeks post-partum the patient had a non-secretory endometrium. It is suggested that this patient's second menstrual cycle was anovulatory in nature and that the dose of Stilbesterol given to control lactation from the breasts was also sufficient to inhibit ovulation in that cycle. All F.S.H. assays on the patient were normal.

One case of persistent amenorrhea at 14-months post-partum is presented (case 10). This patient had two endometrial biopsies, both of which were non-secretory, the first being very atrophic. Both F.H.S. assays were within normal levels (more than 26 Mu and less than 53 Mu per 24-hours).

VI DISCUSSION

In discussing this problem and the above data it is necessary to consider the reclassification of the amenorrheas in the light of our knowledge of F.S.H. which was presented by Albright. There may be primary ovarian failure with hypoestrinism as in the case of menopause patients. In this group F.S.H. is increased in the urine. There may be secondary ovarian failure with hypoestrinism due to pituitary gonadatrophin failure. In this group F.S.H. is decreased in the urine, i.e., Simmond's cachexia. A third group is now established in which there is hypoestrinism with ovarian failure associated with a normal F.S.H. output. There is a psychic factor present in this third group which cannot be overlooked, and it is conceivable that the hypothalamus is responsible in this group for the release of some precursor of estrin via the hypothalamic-pituitary pathway which is non-estrogenic and which is inhibitory to F.S.H. In any case the level of F.S.H. in this class of hypothalamic amenorrheas is normal.

In animals it is established that coitus with subsequent nervous impulses is responsible via the hypothalamic-pituitary tract for the release of luteinizing hormone (LH) necessary for ovulation but whether this is directly applicable to humans is not known. We do know that F.S.H. causes ripening of ovarian follicles, that F.S.H. plus L.H. causes the follicle to produce estrin and to cause ovulation, that L.H. causes corpus luteum formation and that L.H. plus luteotropin causes the corpus luteum to produce progesterone (17).

It then becomes evident to suspect that amenorrhea associated with a normal F.S.H. output may be the result of dysfunction of the hypothalamic-pituitary nervous pathway governing the release of prolactin and L.H.

The predominance of a non-secretory endometrium in contrast to a secretory endometrium may indicate an absence of a functioning corpus luteum as previously speculated. The incidence of non-secretory and secretory endometrium in this series is consistent with that found by Topkins in a larger series.

The incidence of a normal F.S.H. in over 90% of specimens would indicate that this factor of the anterior pituitary is not suppressed during lactation and that lack of ovulation and menstruation cannot be explained on the basis of lack of follicular maturation.

This finding is consistent with the unpublished data supplied by Dr. Anne Forbes concerning 9 assays of F.S.H. done on lactating women in Dr. Fuller Albright's laboratory. All 9 of these assays were within the normal levels as stated herein, (personal communication).

V11 CONCLUSIONS

From this study the only conclusions justified are: 1. that the anterior-pituitary gland output of F.S.H. during lactation is normal in 90% of cases; 2. that there is no relation between this level of F.S.H. and the incidence of menstruation or ovulation during lactation; 3. that the amenorrhea of lactation is not due to failure of the follicle to receive its growth stimulus.

It may be justifiable to suppose that the amenorrhea of lactation may be due to a lack of L.H. or luteotropin and that a hypothalamic-pituitary nervous mechanism may be responsible for this.

V111 SUMMARY

The problem of the amenorrhea associated with lactation is presented, the literature reviewed and the incidence of catamenia discussed. Thirteen cases oflactation amenorrhea, complete and incomplete, are studied and reports of 36 endometrial biopsies and 34 F.S.H. assays are given. The incidence of non-secretory endometrium is found to be 97%, of secretory endometrium 3%. The incidence of normal daily levels of F.S.H. is 91%. Sixty-nine percent of patients were complete amenorrheas and 31% had catamenia re-established during the period of observation. The significance of the observations are considered and it is concluded that the amenorrhea of lactation is not due to suppression of the anterior pituitary follicle stimulating factor.

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