1966

Methods of timing ovulation

Leonard Kenneth Lamberty
University of Nebraska Medical Center

METHODS OF TIMING OVULATION

Leonard Kenneth Lamberty

Submitted in Partial Fulfillment for the Degree of
Doctor of Medicine

College of Medicine, University of Nebraska

March 1, 1966

Omaha, Nebraska

TABLE OF CONTENTS

I. INTRODUCTION

It is a generally accepted fact that ovulation in the human occurs approximately midway between menstrual periods in the "normal" 28-day cycle. Ogino (43), by inspecting the ovaries of patients undergoing laparotomy, concluded that the day of ovulation is more closely related to the succeeding menstruation, occurring 12 to 16 days before it, than to the previous period. Several young human embryos have been discovered within uterine specimens after hysterectomies, and the day of ovulation estimated from their age. These findings are summarized by Hartman (33a), and in general indicate that ovulation occurs at midcycle.

The determination of the exact time of ovulation has been the subject of much research for many years. There are two basic uses that could be made of such information: (1) accurate determination of the "safe period" for couples using the "rhythm" method of birth control; (2) timing of coitus or artificial insemination for couples with an infertility problem. Hartman states that the exact length of time the human ovum is fertilizable is unknown, but an upper limit of 24 hours is generally assigned, based on animal experiments (33b). Thus the fertile period is a maximum of three days in length, and any ovulation test used for infertility cases should be accurate enough to show the day of ovulation plus or minus one day. If a test is used to determine the safe period, it should be capable of predicting the day of

ovulation at least two days beforehand.

Many of the tests used or suggested for timing ovulation involve complicated and expensive laboratory determinations and/or daily visits to the doctor. This may be acceptable in the case of the infertile couple wishing to conceive, but it is obviously not practicable for those wishing to avoid conception. Sturgis (52) states that "the most useful test should (1) show that ovulation is about to occur or that it has occurred within hours, not days, (2) be easy to perform without complicated and expensive laboratory facilities, and (3) lend itself to interpretation by the patient herself for the purpose of coital timing." To be useful in the rhythm method of birth control, as previously stated, the test should predict ovulation at least two days in advance.

The purpose of this paper is to sunnnarize the tests for ovu• lation which have been used clinically or show promise of being useful. Emphasis will be placed on those tests which tend to fulfill the criteria above.

II. ESTABLISHING PROOF OF OVULATION

There are only two methods of absolutely proving ovulation: (1) direct observation of ovulation, and (2) establishment of pregnancy (4). Ovulation has been directly observed on a few occasions (13, 20, 21) using culdoscopy. However, this obviously cannot be used as a routine procedure. Conception is useful as a means of confirming a test for ovulation timing only if it results from isolated coitus or a single artificial insemination.

The presence of a corpus luteum is also generally regarded as proof of prior ovulation. Corner (17) has described in detail the day-to-day histological changes in the corpus luteum so that its presence in surgical specimens may be used to date ovulation within approximately one day. Many investigators have correlated the histological appearance of the corpus luteum with results of various tests for ovulation in their research series on these tests.

The histological appearance of the endometrium has been used in infertility studies primarily to determine whether ovulation has occurred, since the secretory phase is dependent upon progesterone secreted by the corpus luteum. Noyes et al (42) described their criteria for dating the endometrial biopsy. The earliest morphological evidence of ovulation is first seen 36-48 hours after ovulation occurs. Therefore this test, although useful for confirmation of others under investigation, could not be used in itself for timing of coitus. Furthermore, as a routine daily technic,

it is impractical and carries with it the possibility of dislodging an existing pregnancy {4). It is included in this section because of its definite dependence on an intact corpus luteum (and therfore prior ovulation). However, hormone therapy will reproduce these changes, without ovulation having eecun:e4, and thus invalidate the test.

Although many investigators have correlated the test being studied with other indirect tests for ovulation time, an attempt was ma.4e to obtain data using one of the four methods above as confirmation for each test.

III. TIMING OF OVULATION--POTENTIALLY USEFUL METHODS

1. Intermenstrual Pain

The occurrence of "Mittelschmerz" in some women is well-known. Krohn (38) described the regular occurrence of intermenstrual pain in a patient for 9½ years. It was absent only during pregnancy and the first few months of lactation. He did not attempt to correlate it with any other phenomena. Wharton and Henriksen (57) found ruptured follicles with varying amounts of free blood in the pelvis in nine patients who underwent laparotomy during the occurrence of such pain. No one has given a definite explanation for the pain, but Sturgis (52) gives two possible reasons for it: (1) preovulatory distention of the ovarian capsule over the enlarging follicle; (2) postovulatory irritation of the peritoneum from blood spillage. He concludes that until its causes are understood, it has little definitive value other than calling attention to the probability that ovulation has occurred. It cannot be depended on to give the necessary prediction of ovulation for the rhythm method of birth control. Also, since it occurs regularly in only 25 per cent of women (41), other methods are necessary for the majority.

2. Hormone Bioassays

The usual methods of determining hypophyseal and ovarian hormone secretions involve bioassay of the hormones or

their metabolites in the urine. The typical cycle described by D[1].Amour (18) involves a gonadotropin peak at midcycle, a bimodal estrogen peak occurring on either side of the gonadotropin peak, and the appearance of pregnanediol in the last half of the cycle, after the gonadotropin peak. He considered the gonadotropin peak the most indicative of the exact time of ovulation. Since he did his work, Ill has been recognized as a separate entity in the gonadotropins, and it is generally accepted that it must be released in increasingly effective amounts during the two to three days i.nnnediately prior to ovulation (52). Taymor (53) describes a bioassay of urine for LR, using the increased weight of the ventral prostate of hypophysectomized male rats as an index. Correlating his results with examination of corpora lutea obtained in the same cycles as the assays were done, he found a peak of LR excretion occurring 12 to 48 hours before ovulation.

The only bioassay method of timing ovulation which has been used in comparatively large series of women is the rat ovary hyperemia test originated by Farris (26) and often referred to by his name. This test depends on the degree of ovarian hyperemia observed in the ovaries of rats sacrificed two hours after subcutaneous injection of two cc. of first morning urine of the patient being tested. The test is performed daily beginning five to six days prior to the anticipated date of ovulation. Based on results of tests on 100 patients, Farris concluded that a normal reaction

is the occurrence of definite hyperemia for three or four days near the midcycle. Abnormal reactions include lack of hyperemia, only one or two days of hyperemia, or a split reaction with one or two day periods of color separated by one or more days of no response. In his first series, ten patients became pregnant when coitus was performed on the third or fourth day of hyperemia, but no conceptions occurred when coitus was delayed until the color reaction disappeared. Of fifty patients in whom repeated artificial insemination had been unsuccessful, only eleven showed normal reactions. Eight of these conceived when the insemination date was chosen according to the results of the test. No conceptions occurred in patients with abnormal reactions. One patient underwent laparotomy on the second day of the reaction, and a 16 mm. follicle was discovered. Based on these results, Farris concluded that ovulation occurs on or within 24 hours of the last day of the color reaction.

In two subsequent series (27, 28), Farris reported a total of 69 patients who conceived using the rat test to time coitus or artificial insemination. Corner et al (16) correlated the hyperemia test with dating of endometrium and corpus luteum in 39 patients and showed agreement within one day in 26 cases. Differences of two to four days occurred in four cases, and in three cases all tests were negative for ovulation. In the remaining six, the rat test was positive but ovulation had not occurred.

In each, a large follicle was present but had begun to retrogress. This was interpreted as a pathological entity.

Dresner and Cohen (25) reported modifications of the Farris test made by Behrman because several investigators were unable to confirm results obtained by Farris. Using the modified technic, they obtained fairly good correlation with several other tests (which remain to be discussed here) in 69 infertility patients. Correlation was excellent in the ten cases in which conception followed artificial insemination.

Farris tested several pure hormones and concluded that the hyperemia was probably due to increasing LH excretion (26). This view is also held by others (4, 25). However, even though the rat test gives one to two days prediction of ovulation it is not generally recommended for routine ovulation timing (13, 25).

3. Basal Body Temperature Curves

Probably the most widely used method of timing ovulation, for both conception and contraception, is the basal body temperature (B.B.T.) curve. A record of the body temperature, taken under basal conditions (on first awakening in the morning), shows a typical curve during the menstrual cycle. It is relatively low during the first part of the month, drops to a minimum about the time ovulation occurs, and then rises definitely to a relatively high level which is maintained until it drops abruptly at the onset of the next menses. According to Tompkins (54), the important

feature is the rapid rise at ovulation. Rubinstein (46) stated the rise should exceed 0.5 degree F. in the first 24 hours after ovulation and 1.0 degree F. in the first week. However, Siegler and Siegler (50) found that a rise of 0.4 degree F. was an adequate thermal shift. In their series of infertility patients, it required one to eleven days to attain this increase, with a mean of 3.5 days. Of their patients who became pregnant, only 1/3 had a rapid temperature shift (occurring in less than two days), while 2/3 had a slow "staircase" shift.

The temperature must be taken under strict basal conditions (13). Several authors prefer rectal temperatures (13, 31, 46, 50), but Halbrecht (32) found no practical difference in reliability between those taken orally, rectally, or vaginally. It should be taken for at least five minutes at the same time every day, after at least six hours of restful sleep, and at least eight to twelve hours after the last food intake. The patient must note the presence of infection, any interruptions to sleep, nightmares, gastrointestinal distress, or other circumstances that might affect the temperature (46).

The cause of the typical temperature curve has been shown (10, 19, 37) to be caused by the rhythmic production of estrogen and progesterone. Estrogen causes a decrease in temperature, while progesterone, or a combination of estrogen and progesterone, causes increased temperature. These effects were demonstrated in

surgical castrates, normal women, and women with secondary amenorrhea.

The exact time of ovulation in relation to the temperature shift has been the subject of much speculation and research. Rubinstein (46) and Davis and Nicholas (19) theorized that luteinization and minimal progesterone production begin before ovulation and that therefore the temperature begins to rise shortly before ovulation. Isolated coitus on various days of the cycle resulted in twenty pregnancies in Zuck's series (60). The day of coitus ranged from three days before to one day after the initial temperature rise after the low. A high number of the pregnancies occurred with coitus at the time of the low temperature. The Sieglers (50) achieved eight conceptions with single inseminations. They ranged from three days before to two days after the initial temperature rise, with five of the eight conceptions occurring within one day of the low temperature (one day before the rise). Barrett et al (3) recorded fourteen conceptions, all of which occurred with coitus at or before the initial rise. Doyle (21) observed the ovaries of seven patients at culdotomy done on the day of low temperature. In two, follicles were seen to rupture six and thirty hours after the low point. Hemorrhagic atretic follicles were seen in three, and early corpora lutea were seen in two. Greulich (31) performed laparotomies on several inmates of a home for feeble-minded. He correlated the microscopic dating of the corpus luteum, if found, with the B.B.T. and concluded

that in most women, ovulation occurs after the temperature begins to rise. However, some of his cases indicated that ovulation had probably occurred before the temperature rise. Buxton and Engle (11) performed elective operations involving laparotomy on the morning of the day of initial temperature rise. Of eighteen patients, six had not yet ovulated, while the corpora lutea from those who had, ranged up to 72 hours postovulatory. They cautioned that in determining ovulation using B.B.T., there may be an error of nearly 24 hours, because ovulation conceivably can occur at any time, while temperature is taken only once a day. They concluded that using this method may produce an error of as much as four days in determining ovulation time.

Noyes et al (42) found good correlation between the B.B.T. and endometrial biopsy, with a tendency for the low point to fall on the day before progesterone dominance in the endometrium. Taymor (53) compared the B.B.T. curve with LH bioassay in ten women. In five, the peak LH excretion coincided with the low temperature; in three, the peak LH excretion preceded the low point by 24 hours; and in two, the peak LH excretion occurred 48 hours after the low point on the temperature curve. He concluded that either the peak LH excretion or the thermal shift on the B.B.T. curve is not in a consistent time relation with ovulation. Farris (28) used the rat ovary hyperemia test to determine the date of ovulation in 27 patients who conceived, comparing this with the

B.B.T. curve. He found that 41 per cent of the conceptions occurred at the low, during the rise, or after the rise. He, along with others (4, 13, 18), concluded that B.B.T. changes are not reliable enough to be considered an accurate method of timing ovulation.

4. Vaginal Smears

Serial vaginal smears, done daily, have been used, along with other tests, to time ovulation in infertility patients (4, 13). Rubinstein (46) describes and illustrates the cells found in such smears and details the changes seen during the menstrual cycle. Estrogen causes cornification of vaginal epithelial cells, and therefore maximum cornification occurs slightly before ovulation. Progesterone causes desquamation of the superficial cells and appearance of more immature cells in the smear. D'Amour (18) states that the day-to-day changes are so gradual that no one day is sufficiently distinctive to indicate ovulation. However, Behrman (4) found vaginal smears extremely useful, with a sharp change visible on the day of ovulation. Vaginal smears cannot be used in the presence of cervicitis or vaginitis and are difficult to interpret following coitus (13). Another disadvantage is that the long training and experience of an expert cytologist are required to interpret the changes incident to ovulation (4, 14).

5. Occult Intermenstrual Bleeding

Gross intermenstrual bleeding has been known to occur in a certain percentage of women for many years. This is

generally considered too unreliable to be depended upon as a sign of ovulation (4, 13). However, Bromberg and Bercovici (9), using the benzidine test on vaginal secretions, detected intermenstrual bleeding in 75 of 80 patients, all of whom ovulated as shown by endometrial biopsy. They concluded that the bleeding occurs early after ovulation (1-4 days), based on twelve endometrial biopsies done on the day of onset of bleeding, three corpora lutea found on laparotomy 1-2 days after bleeding began, and three pregnancies resulting from isolated coitus 1-2 days before bleeding began. Large doses of stilbestrol were found to inhibit both ovulation and intermenstrual bleeding in most cases. In some, bleeding, but not ovulation, was inhibited, suggesting that the bleeding is not directly due to ovulation. They raised the possibility of its resulting from an estrogen withdrawal effect.

On the other hand, Hurtig (36) suggested that the blood comes from the ovary, causing a positive test in the vaginal secretion several hours after ovulation. He used Hematest and Occultest tablets to test vaginal tampons which had been inserted daily for 30 minutes. Using 80 healthy volunteers and a total of 218 cycles, he found a sharply defined episode of intermenstrual bleeding in 162 cycles or 74.3 per cent. There was persistent postmenstrual bleeding in 15 cycles, and eight patients had continuous positive results. Three of these had lesions (two fibroids and one endometrial polyp), and three others had been

treated for iron-deficiency anemia for several years but had no lesions. Ten patients had consistently negative tests for blood. Only one of them had any children, although the others were all married for at least two years. Hurtig gives no record of correlations with other tests for ovulation except in the case of seven infertility patients who had positive tests for intermenstrual bleeding and demonstrated secretory endometrium premenstrually at biopsy. He does suggest observation of the ovaries by laparotomy or culdoscopy on the day of onset of bleeding.

Barrett et al (3) failed to demonstrate any definitive midcycle peak in vaginal bleeding that could be correlated with the thermal shift of the B.B.T. They did find premenstrual and postmenstrual peaks in a large number of cases.

In contrast to the two explanations of intermenstrual bleeding offered above, Hartman (33d) states that it is due to the high estrogen levels in the blood and is analogous to the period of "heat" in lower mammals.

6. Physical Changes in Cervical Mucus

a. General

The cervical mucus undergoes cyclic changes associated with alterations in sperm penetrability. In the postmenstrual period, the mucus is scant, viscous, and sticky, and contains many polymorphonuclear leukocytes. Sperm penetrability is low. In the immediate preovulatory phase, and synchronous with the "ovulatory dip"

in the B.B.T. curve, there is a definite increase in volume, a marked decrease in viscosity, and practically complete absence of polymorphonuclear leukocytes. It is easily and rapidly penetrable by relatively large numbers of sperm, which remain actively motile for 24-72 hours, longer than at any other time in the cycle. These conditions prevail for only one to three days in the average woman's cycle. After ovulation, and with the establishment of the rise in the B.B.T. curve, the volume decreases and viscosity increases rapidly, and leukocytes are again present. Sperm again penetrate poorly (1).

Viergiver and Pommerenke (55) performed daily quantitative measurement of the mucus and correlated this with the B.B.T. curve in seven cycles. They found a relatively constant quantity until the eighth or ninth cycle day, a marked increase (at least three-fold) for about four days, then an abrupt decrease to approximately postmenstrual levels, where it remained for the rest of the cycle. In six cycles the maximum secretion preceded the temperature rise by one to three days, while in the seventh the maximum secretion occurred on the same day as the rise in temperature.

Bergman (5) measured the dry content of the mucus and defined the "water phase" as the period when the dry content is less than seven per cent. This approximately coincides with the time of maximum estrogenic secretion before the temperature shift.

Abarbanel (1) duplicated the preovulatory mucorrhea in seven female castrates, who had only a cervical stump remaining,

by administration of various estrogens. Progesterone and testosterone preparations did not cause the changes. The postovulatory changes in mucus were reproduced in estrogen-primed castrates by administration of progesterone.

b. Spinnbarkeit

At the time the cervical mucus increases in volume, its ability to be stretched into threads (Spinnbarkeit) also increases. The use of this property in timing ovulation requires that mucus be aspirated from the cervix daily, placed on a clean glass slide, and covered with a cover slip. The cover slip is then withdrawn, and the maximum length of the mucus thread thus formed is measured in centimeters. Usually the measurement is repeated a few times and the average value used (14). Spinnbarkeit is minimal in the postmenstrual period, begins to increase with the preovulatory mucorrhea, and reaches a maximum of five to twenty centimeters during the fertile period (15). After ovulation, it becomes minimal or disappears completely.

Cohen et al (14, 15) have found good correlation of maximal Spinnbarkeit with the B.B.T. curve and serial vaginal smears. It usually precedes or coincides with the temperature shift. In a series of 23 patients who became pregnant after isolated artificial insemination, 18 were inseminated on the day of maximal Spinnbarkeit, 4 within one day of the maximum, and one the second day after it.

Cohen and Hankin (13) believed that maximal Spinnbarkeit is the best indication of optimal fertility and stated that patients who ovulate without cervical mucorrhea and Spinnbarkeit are infertile. Behrman (4) agreed that it is a "valuable, easy and practical" test for ovulation.

c. Crystallization (The Fern Test)

Papanicolaou (44) was the first to describe a "secretion" in the vaginal smear which stained brown or yellow with silver nitrate. When abundant, it "tends to spread in the form of anastomosing branches with heavily indented projections. At other times it appears in round droplets of various sizes in single or bifurcated rods or in starlike formations." This "secretion" becomes very typical and conspicuous at about the time of ovulation. It decreases and practically disappears at other times in the cycle, during pregnancy, in the presence of amenorrhea, and after the menopause. It increases after the administration of estrogenic hormones.

Papanicolaou also found his "argyrophil secretion" in the cervical mucus in larger amounts and at all times, with a variation in the quantity and form. Rydberg (47) found that the brown-staining substance was sodium chloride, the brown color being due to the reduction (by oxygen in air) of silver chloride to free silver. He described microscopic crystal formations in dried cervical mucus smears as resembling fern or palm leaves. The

crystals are sodium chloride, and the typical formations are due to the presence of mucin. The amount of crystallization varies widely during the menstrual cycle and is maximal in the preovulatory and ovulatory period.

Bergman (5) found that the typical cyclic changes can be induced by administration of estrogen and progesterone. Estrogen causes maximum crystallization, while progesterone causes it to decrease or disappear. Zondek and Rozin (59) confirmed this and found that the crystallization was directly due to electrolytes in the mucus. Using mucus with absent or poor ferning, they produced the typical pattern by the addition of not only sodium chloride, but also potassium chloride and potassium bromide. Arborization is inhibited by the presence of semen, blood, or serum in the mucus. They concluded that examination of the cervical mucus for ferning at midcycle and again in the premenstruum could be used to confirm the occurrence of ovulation in the cycle tested.

Forman (29) undertook to describe in detail the typical changes in the crystallization pattern. He found ferning most marked in days 12-16 of a typical 28-day cycle, when the mucus showed maximal Spinnbarkeit, and believed this indicated imminent or recent ovulation. Peak arborization was observed to precede by a day or two the typical drop in the B.B.T. curve which he considered to coincide with ovulation. He concluded that daily examination of the mucus combined with the B.B.T. record is more

accurate in timing ovulation than the B.B.T. alone.

Roland (45) emphasized that atypical ferning must be differentiated from the true fern pattern in order to use the test. The presence of blood or any cervical infection will inhibit true fern formation. He did not believe there was enough day-to-day change in the pattern to permit singling out one smear during the cycle as indicative of ovulation.

Cohen and Hankin (13) felt that the fern phenomenon usually extends over too long a period of time for usefulness. However, it may be of value in the occasional patient who has poor mucorrhea and shows a positive fern test for only one or two days.

Ferning has been reported in nasal mucus (59) and in saliva (47), but the patterns are not so marked as in cervical mucus. Although Zondek and Rozin (59) reported that it was present at all times in nasal mucus, Henderson (34) found cyclic changes similar to those in cervical mucus. Andreoli and Porta (2) also found typical cyclic changes in saliva.

7. Chemical Changes in Cervical Mucus

a. Glucose

Viergiver and Pommerenke (56) found that the cervical mucus contained free reducing substances throughout the menstrual cycle and that these substances were present in lowest concentration at the time of ovulation.

Birnberg et al (7) suggested that the reducing substance might be glucose derived from the hydrolysis of glycogen. They performed daily tests for glucose on normal women, using a strip of Tes-Tape placed in the cervical canal. Beginning about two days before the theoretical day of ovulation, they detected a faint color change which reached maximum intensity on the theoretical day of ovulation, then faded and disappeared within two or three days. They performed the test on several patients admitted for elective hysterectomy on the day before surgery. Ten of the patients had a positive reaction, and in all ten, fresh or recent hemorrhagic corpora lutea were found. In 27 patients, artificial insemination was performed on the first day of deep color change. Twenty patients (74%) became pregnant after the first insemination. Three others conceived on subsequent attempts.

To make the test easier to perform, they devised wooden and plastic applicator sticks with cotton tips impregnated with the same reagents used in Tes-Tape. To rule out the possibility of some substance other than glucose causing the reaction, applicators without glucose oxidase were used in parallel tests. They gave no color change, even when a positive reaction was recorded with glucose oxidase present.

Cohen (12) compared the glucose reagent stick test with the B.B.T. curve, vaginal smears, and Spinnbarkeit, during 95 cycles in 63 infertility patients. He found fairly good correlation of the

positive stick test with maximal Spinnbarkeit and vaginal smear cornification and extremely poor correlation with the temperature shift. He found that ovulation can occur despite negative stick tests as shown by pregnancy resulting from artificial insemination in three patients with consistently negative stick tests. He concluded that the occurrence of glucose is physiologic, being necessary for the nutrition of spermatozoa, but that the stick test is an indication of fertility potential rather than a test simply of ovulation. He suggested that daily observations using a battery of tests should prove more reliable in timing ovulation than any one test.

Siegler (49) used the glucose reagent sticks to test for cervical and vaginal glucose and compared the results with B.B.T. graphs in 20 patients and endometrial biopsies in 14 of these. He concluded that there was no phasic tendency or consistent alteration in the intensity of the color reaction which could pinpoint the time of ovulation, and he found no definite relation between the glucose color reaction and the B.B.T. or the phase of the endometrium. These latter two criteria compared favorably with each other. He found some positive glucose tests in the pre-ovulative and progestational phases of the cycle and occasional negative tests during the thermal shift. However, all of his subjects were infertility problems, and none underwent daily testing for glucose throughout the cycle, as suggested by the originators of the test.

Birnberg et al (8) improved the sensitivity and usefulness of the test by using applicators with a more sensitive glucose oxidase system (Ovutest). Using Ovutest, they observed a general correlation between vaginal and cervical glucose but found the vaginal peak occurred more often one or two days before, and only occasionally one day after, the cervical peak. The typical vaginal Ovutest chart showed a rise at the ovulatory phase and a secondary peak at about the twenty-fifth day of the cycle, which coincides with increased Spinnbarkeit at this time. They concluded that the vaginal test is useful in detecting the ovulation phase but the cervical test is usually more precise in pinpointing ovulation.

Of 20 patients examined by culdoscopy on the day of maximum glucose (by Ovutest), 17 (85%) showed evidence of fresh ovulation. Of the others, two had microcystic ovaries and one had an ovarian cyst.

It was found that in postmenopausal women, the glucose reaction could be intensified by exogenous estrogens and diminished by large doses of progesterone. Therefore, it is assumed that the glucose test parallels the level of estrogen with a peak just before or coincident with ovulation.

Doyle (22) first used a strip of Tes-Tape paper over the tip of a cardboard or plastic-covered tampon to test daily cervical glucose concentration. He found the maximum color response to occur simultaneously with the appearance of two early corpora

lutea by culdotomy and laparotomy. The test was positive at the same time on follicular fluid and on aspirate from the cul de sac. Therefore, he believed the cervical glucose reaction may be due to aspirated liquor folliculi. Later, with Ewers (23), he modified this view and concluded the cervical glucose comes mostly from glycolysis within the cervix. This was based on positive glucose tests on a woman who had undergone supracervical hysterectomy and bilateral salpingectomy without oophorectomy. Birnberg et al (8) presented a patient who had had a total hysterectomy and left salpingo-oophorectomy. She gave a markedly positive vaginal glucose test prior to her second laparotomy, at which a fresh corpus hemorrhagicum was found. This provides evidence that vaginal glucose is "intrinsic" and not derived from follicular fluid or cervical mucus.

Doyle and Ewer (23) designed the "Fertility Testor", a syringe-like instrument inserted into the vagina to provide contact of the Tes-Tape with the cervix but not with vaginal secretions. Results with this were the same as those obtained using the tampons. Because Tes-Tape contains the dye orthotolidine, which chemically resembles the carcinogen bensidine, a new tape was developed using a different dye, which is approved by the F.D.A. (24). Tes-Tape occasionally has shown sporadic irregular positive reactions, but the new tape has no such action. It is used in the Fertility Testor as Tes-Tape, but the color change is from pink to pale blue in the presence of glucose.

Using the Fertility Testor, the initial appearance of glucose was found to usually precede the temperature shift by one or two days, but occasionally by up to five days; it has also occurred up to four days after the shift. Of 40 infertile women who underwent culdotomy or laparotomy, 35 showed a positive glucose reaction and were found to have rupturing or ruptured follicles or early active corpora lutea. The remaining five had negative glucose reactions and showed no evidence of ovulation. Two instances of double ovulation from the same ovary in the same cycle were photographed. A double wave of glucose had been observed, but the double ovulation had not been suggested by the B.B.T. curve. The second ovulation occurred one to three days after the first.

Two advantages of the glucose test over B.B.T. are that activity during the night has no effect on the glucose test, and its interpretation by the patient is easier.

The glucose test may be of limited value in diabetics and the occasional patient who shows a strong positive throughout the cycle, but these patients, and those with bacterial, trichomonal, or monilial vaginitis, usually can get satisfactory results if the test is preceded by a hot water douche and drying of the vagina with a tampon. Cervical erosions and polyps will minimize the glucose concentration.

The authors suggest that in using the test with the object of pregnancy, coitus be performed on the first day of the positive

result. To avoid pregnancy, they suggest abstaining after menstruation and until four days after the test has become negative.

Cohen and Hankin (13) correlated results of the Fertility Testor with several other criteria of ovulation, including B.B.T. curve, cervical mucorrhea, Spinnbarkeit, ferning, and the vaginal smear. In general, correlation was only fair, and they did not feel it offered any advantages over their other tests.

Mier and Ostolaza (41) felt there were many disadvantages in the use of the Fertility Testor and believed that unsatisfactory results might be due to the difficulty of blindly inserting the instrument in the hope of testing only the cervical mucus.

Barrett et al (3) tested for cervical glucose by having their patients insert a tampon for at least 30 minutes, then withdraw it and test it with Clinistix. When present, the glucose showed two peaks, the first approximately at the time of ovulation, and the second, not as pronounced as the first, about one week before menstruation. They considered the second peak to correspond to the "premenstrual or second estrogen peak of the normal monthly cycle." They found fairly good correlation of the positive glucose tests with the temperature shift (228 of 308 cycles).

b. Sodium Chloride

Since the fern test depends on crystallization of electrolytes (primarily sodium chloride) in the mucus, McSweeney and Sbarra (39, 40) investigated the chloride changes occurring during the cycle and developed a simple test to detect these changes.

The test paper is prepared by serially impregnating filter paper sheets with solutions of silver nitrate and potassium chromate, resulting in a brown precipitate of silver chromate. Any chloride ions placed on this paper will result in a white to buff-colored spot, the intensity of which is roughly proportional to the amount of chloride present. A set of standards showing the color changes given by solutions ranging from 0.1 per cent to 1.0 per cent sodium chloride is used for comparison with the test paper.

If the physician performs the test, he takes a smear of mucus directly from the cervical canal with a cotton swab and rubs it thinly on the test paper. Within a few seconds, the color change is compared with the standards to determine the sodium chloride concentration.

The patient can take smears herself using a cotton-tipped plunger in a cardboard or plastic cylinder, which is introduced well up into the vagina. The plunger is pushed out to contact the cervical mucus and is then withdrawn into the cylinder before the device is removed. The cotton tip is then rubbed over a small area of the test paper.

The typical menstrual cycle shows a low chloride concentration (less than 0.3%) postmenstrually. The concentration increases in the two to eight days before the presumed time of ovulation. The peak chloride concentration, approximately 0.9 per cent is assumed to occur with ovulation. It then decreases to less than 0.5 per cent on the following day and remains low until menstruation.

In order for accurate results to be obtained, the cervix must be clean and free from infection, and there must be no vaginitis. Deep cauterization with destruction of most of the endocervical glands will weaken or nullify the test.

This test has correlated well with the fern test, Spinnbarkeit, vaginal smears, and the B.B.T. curve. Maximal ferning, Spinnbarkeit, and vaginal cornification occur at the time of maximum chloride concentration. The temperature rise occurs simultaneously with the decrease in chloride.

Administration of estrogen to castrates and postmenopausal women was shown to cause increased chloride concentration. Progesterone is assumed to cause a decrease, but no tests to confirm this were reported. Also, there was no report of correlation of the chloride test with dating of corpora lutea or pregnancy achieved with isolated coitus or insemination.

In their second communication (40), the authors felt that the test indicates only the "apparent concentration" of chloride and conceivably may be a reflection of the "availability" of mucus for testing. A short time earlier, Herzberg et al (35) had reported that the sodium chloride concentration of fresh cervical mucus is nearly constant and isotonic throughout the cycle. However, the per cent of sodium chloride in dried mucus has a cyclic variation with a very sharp maximum at the time of ovulation.

Nasal mucus was found to show cyclic variations in apparent chloride concentration, using the spot test, when the test was

performed by physicians. However the test was unsatisfactory when performed by the patient at home. Coryza, sinusitis, and allergies render the test useless.

8. Sevag-Colton Colorimetric Test

Sevag and Colton (48) reported a "simple, clinically applicable chemical method for the determination of ovulation time." Urine samples are collected from 11:00 P.M. to 7:00 A.M. for five to seven consecutive nights, beginning with the sixth or seventh day after midnight of the first day of menstruation. The urine samples are tested by Folin-Ciocalteu reagent for the presence of chemical substances which give a blue color suitable for colorimetry. The color-yielding substance is a component of a complex containing a derivative of folic acid. Graphing the intensity of the blue color each day results in a curve with a characteristic peak. The terminal low value, if it occurs on either the fifth or sixth day of the pattern, represents the day of ovulation.

In a series of patients who became pregnant after isolated coitus or artificial insemination, there were 112 conceptions using this test alone to choose the day. Another 37 patients became pregnant after they were tested by both the Farris rat test and the colorimetric test, but the day for insemination was selected by the results of the colorimetric test. No correlation could be found between the two tests.

Behrman (4), although conceding that elaborate laboratory facilities are necessary, felt that this test is one of the few which enables a prediction of ovulation time. He cited one patient who became pregnant after artificial insemination and in whom there was excellent correlation between this test and B.B.T., Spinnbarkeit, vaginal smears, and the Farris test.

Speck et al (51) compared the Sevag-Colton test with B.B.T. and Spinnbarkeit in 34 cycles of 19 women. They found only a 47 per cent agreement between the three tests. Of four pregnancies which occurred, "in only one could the Sevag-Colton test alone have possibly contributed to the success in determining ovulation". They concluded that the colorimetric test held no advantage over B.B.T. or Spinnbarkeit, since it was not superior in accuracy and was less practical to perform.

9. Immunochemical Testing for LH

Wide (58) developed a pregnancy test involving inhibition by the patient's urine (containing HCG) of a hemagglutination reaction between HCG-coated red cells and anti-HCG serum. He discovered that the reaction could also be inhibited by concentrates of human pituitaries and of urine from men and non-pregnant women. In comparing the biological activity of the various pituitary preparations (using various bioassays) with their ability to inhibit the reaction, he found a significant correlation of immunological activity with LH activity but not with FSH or GH activity.

He further discovered that the immunological activity of urine concentrates of non-pregnant women of fertile age was highest at midcycle and lowest during menstruation. He concluded that this activity was due to LH in the urine. Since the urine would not inhibit the reaction unless it was concentrated, there was no interference with the pregnancy test using unconcentrated urine.

Bermes et al (6) found that by using a five-fold concentration of urine, a positive hemagglutination inhibition was obtained for two to four days in the average cycle. They diluted the specimens to determine which had the highest LH titer for comparison with B.B.T. curves and vaginal smears. They assumed that ovulation occurred at about the time of the temperature shift and on the day, or one or two days after, the maximum cornification in the vaginal smear.

In 57 of 59 cycles there was a midcycle elevation of LH. Of the 57, the temperature shift was inconclusive in 6. Thus the maximum LH titer and the B.B.T. shift could be correlated in 51 cycles. In 30 of these the temperature shift occurred on the same day, and in 8 the temperature shift occurred the day after the LH peak. In the other 6 cycles, the temperature shift occurred three to eight days before or after the LH peak.

Daily vaginal smears were taken in only four cycles. In two of these, the LH peak was seen two days after maximum cornification, in one it occurred one day after the maximum, and in one it occurred one day before maximum cornification.

"Ovulation pain" was noted in eight cycles, and in six of these it occurred within 24 hours of the peak titer of urinary LH.

Goss and Taymor (30) developed a latex-HCG agglutination system in which they measured human pituitary LH by its ability to inhibit the reaction and compared it with bioassays of the same preparations. They found excellent correlation between the two methods. However, comparable assays, using human urinary preparations (Pergonal) containing only a small amount of LH, showed only grossly similar relative potencies. They postulated that the high proportions of FSH present in the Pergonal may have affected the bioassay.

IV. SUMMARY

In the "normal" female with a 28-day menstrual cycle, ovulation occurs approximately midway between periods. It is desirable to determine the exact day of ovulation for use in two types of patients: (1) those using the rhythm method of birth control, and (2) those with infertility problems. Since sperm are able to fertilize the ovum for up to 48 hours, any test for use with the rhythm method should predict ovulation at least two days before it occurs. Since the ovum is fertilizable for not more than 24 hours, any test used for infertility patients must become positive earlier than one day after ovulation occurs. The ideal test should be inexpensive, and easy to perform and interpret by the patient herself.

The two absolute proofs of ovulation are direct observation and establishment of pregnancy. Pregnancy can be used to correlate with tests for ovulation timing only if it results from isolated coitus or a single artificial insemination. The presence of a corpus luteum also shows prior ovulation, and its age can be determined within one day. The histological appearance of the endometrium can indicate whether ovulation has occurred, and it has been dated according to time since ovulation. Other tests for timing ovulation should be correlated with at least one of these four, preferably one of the first three mentioned.

Mittelschmerz occurs in only 25 per cent of women. Since its cause is not understood, its exact time relationship to ovulation is unknown. It has not been shown to predict ovulation and therefore is useless in birth control. It might conceivably be used to time coitus if pregnancy is desired.

Hormone bioassays in general are too expensive and time-consuming for routine use. They have been useful in showing the normal cyclic changes in hormone levels. Both LH and estrogen show peak levels at the time of, or shortly before, ovulation. Thus tests based on effects of either of these might be expected to predict ovulation.

The Farris rat ovary hyperemia test is quite accurate and predicts ovulation two or three days in advance. It has the disadvantage of other bioassays in that it is expensive and time-consuming. It would be applicable only to patients with infertility problems.

The basal body temperature (B.B.T.) shows a typical cyclic variation and is widely used clinically. However, it has been shown that the temperature shift does not bear a constant time relationship to the occurrence of ovulation. Also the curve is frequently difficult to interpret until several days after ovulation has occurred. It is subject to error from many sources which are difficult to control. Several authors do not consider B.B.T. changes to be reliable enough for accurate ovulation timing.

There is disagreement over the usefulness of serial vaginal smears in timing ovulation. Since they require daily office visits and must be read by an expert cytologist, the expense would tend to be great. They might be justified in an occasional case of infertility.

Occult intermenstrual bleeding has not been shown to occur uniformly in normally menstruating women. Its cause is subject to disagreement, and it apparently occurs up to four days after ovulation. Therefore it is worthless as a test of ovulation for patients who desire to either avoid or achieve pregnancy.

Cervical mucorrhea, increased Spinnbarkeit, and ferning occur together and are dependent on increasing estrogen levels. They are generally accepted as indicators of the period of optimal fertility and thus are useful in patients who desire pregnancy. They are not useful in the rhythm method because they require daily office visits and because they have not been shown to predict ovulation.

Cyclic changes in cervical mucus glucose concentration have been well-correlated with the "direct" indicators of ovulation. It usually gives a two-day prediction of ovulation by the gradually increasing color change. Since the cervical glucose peak coincides more closely with ovulation than the vaginal peak, the cervical test would be preferable in women desiring pregnancy. There is not complete agreement that the Fertility Testor contacts only the

cervical mucus when used by the patients, so that for greatest accuracy it might be desirable to have the physician perform. the test daily in these women. Since the vaginal peak usually occurs one or two days before the cervical peak, the vaginal test might provide sufficient prediction of ovulation for most women who want to avoid pregnancy. If the Fertility Testor is shown to perfona its intended function (testing only the cervical mucus, it can be used, Possibly with parallel vaginal tests as a roµgh check, by these women.

The spot test for changes in apparent concentratiQn of chloride in the cervical mucus shows promise of being a fairly accurate test for ovulation with adequate prediction of its occurrence. However, as yet no reports have been published of correlation with corpora lutes or pregnancy after isolated insend-nation. This should be done before it can be accepted as an accurate teat.

The Sevag-Colton colorimetric test has shown no advantage over several other tests and has the disadvantage of requiring elaborate laboratory facilities. It is reported to be one of the few tests allowing predicticm of ovulation. However its poor correlation with several other tests raises some question as to its accuracy or its ability to be duplicated by workers other than its originators.

Innnunochemical testing for LH is promising but needs more work before it can be considered a practical test. No correlations have been reported except with B.B.T., Mittelschmerz, and vaginal smears. It certainly deserves investigation in relationship to isolated insemination and/or corpora lutea. It would also be desirable to increase the sensitivity of the reaction so that unconcentrated urine could be used. If urine concentration were not required, the test could probably be simple enough to be performed and interpreted by the patient.

V. CONCLUSION

As of this writing, there has been no accurate, inexpensive test for ovulation which can be performed and interpreted by the patient herself and which predicts ovulation at least two days before it occurs. The testing, by various methods, for cervical (and/or vaginal) glucose is nearest to these ideals. However, it is not accepted by all, so that it should not be considered the ultimate answer. Immunochemical reactions involving LH may be far superior after further testing and modifications. The spot test for chloride is also promising and deserves further work.

The most important reason for the development of the ideal test is the need for accurate prediction of ovulation time in women using the rhythm method of birth control. Until very recently, the only test available to them was the B.B.T. curve, which leaves much to be desired. The infertility patient, although she would benefit from development of the "ideal" test, is in a different situation. Spinnbarkeit, ferning, vaginal smears, and the Farris test can all be used, if necessary, since she will (hopefully) need testing fo a relatively short time until she becomes pregnant. The expense and time required for daily office visits and/or laboratory determinations might be considered worthwhile by the patient if she achieves the desired goal of pregnancy.

VI. BIBLIOGRAPHY

1. Abarbanel, A.R., Artificial Reproduction of the Cyclic Changes in Cervical Mucus in Hwnan Castrates, Trans. Amer. Soc. Stud. Steril. 1946, pp. 46-62.

2. Andreoli, Cesare and Porta, M.D., Cyclic Changes in Female Saliva, J. Clin. Endocr. & Metab. 17:913-914, 1957.

3. Barrett, T.J., Brown, M.A., Eichner, Edward and Morabito, J.F., Diagnostic Tests for Family Spacing: A Clinical Analysis, J. Int. Col. Surg. 36:622-632, 1961.

4. Behrman, s.J., Detection of Ovulation, Postgrad. Med. 27:12•17, 1960.

5. Bergman, Per, Sexual Cycle, Time of Ovulation, and Time of Optimal Fertility in Women, Acta. Obstet. Gynec. Scand. 29:Suppl. 4, 1950.

6. Bermes, E.W. Jr., Heffernan, B.r. and Isaacs, J.H., J.mmuno-chemical Detection of Ovulation, Obstet. Gynec. 25:792-796, 1965.

7. Birnberg, C.H., Kurzrok, Raphael and Laufer, Anne, Simple Test for Determining Ovulation Time, J.A.M.A. 166:1174-1175, 1958.

8. _____, Wexler, David and Gross, Milton, Estimation of Ovulation Phase by Serial Tests of Cervical and Vaginal Glucose, Obstet. Gynec. 21:194-200, 1963.

9. Bromberg, Y.M. and Bercovici, B., Occult Intermenstrual Bleeding About the Time of Ovulation, Fertil. Steril. 7:71-79, 1956.

10. Buxton, C.L.· and Atkinson, w.B., Hormonal Factors Involved in the Regulation of Basal Body Temperature During the Menstrual Cycle and Pregnancy, J. Clin. Endocr. 8:544-549, 1948.

11. _____ and Engle, E.T., Time of Ovulation, Amer. J. Obstet. Gynec. 60:539•551, 1950.

12. Cohen M.&., Glucose Reagent Stick Test Compared with Other Criteria for Detection of Ovulation, Fertil. Steril. 10:340-349, 1959.

13. _____and Hawnkin, Henry, Detecting Ovulation, Fertil. Steril. 11:497-507, 1960.

14. _____, Stein, I.F., Sr. and Kaye, B.M., Spinnbarkeit: A Characteristic of Cervical Mucus, Fertil. Steril. 3:201-208, 1952.

15. _____ and _____ Optimal Time for Therapeutic Insemination, Fertil. Steril. 7:141-154, 1956.

16. Corner, G.w., Sr., Farris, E.J. and Corner, G.w., Jr., The Dating of Ovulation and Other Ovarian Crises by Histological Examination in Comparison with the Farris Test, Amer. J. Obstet. Gynec. 59:514-528, 1950.

17. Corner, G.w., Jr., The Histological Dating of the Human Corpus Luteum of Menstruation, Amer. J. Anat. 98:377•392, 1956.

18. D'Amour, F.E., A Comparison of Methods Used in Determining fhe Time of Ovulation, J. Clin. Endocr. 3:41-48, 1943.

19. Davis, M.E. and Fugo, N.w., The Cause of Physiologic Basal Temperature Changes in Women, J. ciln. Endocr. 8:550-563, 1948.

20. Decker, Albert, Culdoscopic Observations on the Tubo•Ovarian Mechanism of Ovum Reception, Fertil. Steril. 2;253-259, 1951.

21. Doyle, J.B., Exploratory Culdotomy for Observation •f Tubo• Ovarian Physiology at Ovulation Time, Fertil. Steril. 2:475-486, 1951.

22. ____ ,- Cervical Tampon--Synchronous Test for Ovulation, J.A.M.A. 167: 1464-1469, 1958.

23. _____ and Ewers, F.J., The Fertility Testor, .1.k.»;.A(. 170:45-46, 1959.

24. _____ .,·Ewers, F.J., Jr. and Sapit, Donald, The New Fertility Testing-Tape: A Predictive Test of the Fertile Period J.A.M.A. 172:1744•1750, 1960.

25. Dresner, M.H. and Cohen, M.R., Ovulation Time: A Modified Rat Hyperemia Test Compared with Other Criteria, Amer. J. Obstet. Gynec. 80:1049-1053, 1960.

26. Farris, E.J., A Test for Determining the Time of Ovulation and Conception in Women, Amer. J. Obstet. Gynec. 52:14•27, 1946.

27. The Prediction of the Day of Human Ovulation by the Rat Test as Confirmed by Fifty Conceptions, Amer. J. Obstet. Gynec. 56:347-352, 1948.

28. ____ Temperature Compared with Rat Test for Prediction of Human Ovulation, J.A.M.A. 138:560-563, 948.

29. Forman, Isador, Cervical Mucus Arborization, Obstet. Gynec. 8:287-292, 1956.

30. Goss, D.L. and Taymor, M.L., Measurement of LR activity by Latex-HCG Agglutination Inhibition, Endocrinology 71:321-324, 1962.

31. Greulich, W.W., The Reliability of "Basal" Body Temperature Changes as an Index of Ovulation in Women, Trans. Amer. Soc. Stud. Steril. 1946, PP• 76-97.

32. Halbrecht, I., Further Observations on the Basal Temperature in Sterile Women, J. Obst. & Gynaec. Brit. Emp. 54:848-852, 1947.

33. Hartman, C.G., Science and the Safe Period, Baltimore, Williams & Wilkins, 1962. (a) P• 199, (b) P• 69, (c) PP• 73-74, (d) P• 180.

34. Henderson, I.D., Cyclical Changes in Female Nasal Mucus, J. Clin. Endocr. & Metab. 16:905-906, 1956.

35. Herzberg, M., Joel, C.A. and Katchalsky, A., The Cyclic Variation of Sodium Chloride Content in the Mucus of the Cervix Uteri, Fertil. Steril. 15:684-694, 1964.

36. Hurtig, Abraham, Occult Uterine Bleeding, Canad. M.A.J. 78:681-685, 1958.

37. Israel, S.L. and Schneller, Oscar, The Thermogenic Property of Progesterone, Fertil. Steril. 1:53-64, 1950.

38. Krohn, P.L., Cyclical Intermenstrual Pain: the Mittelschmerz, J. Clin. Endocr. & Metab. 1 :682-684, 1954.

39. McSweeney, D.J. and Sbarra, A.J., A New Cervical Mucus Test for Hormone Appraisal, Amer. J. Obstet. Gynec. 88:705-709, 1964.

40. ▬▬▬ and A Rapid Ovarian Hormone and Ovulation Test, Obstet. Gynec. 26:201-206, 1965.

41. Mier, T.M. and Ostolaza, M.F., The Time of Ovulation in Women, Missouri Med. 60:138-140, 1963.

42. Noyes, R.W., Hertig, A.T. and Rock, J., Dating the Endometrial Biopsy, Fertil. Steril. 1:3-25, 1950.

43. Ogino, Kyusaku, Conception Period of Women, Harrisburg, Pa., Medical Arts Publishing Co., 1934.

44. Papanicolaou, G.N., A General Survey of the Vaginal Smear and its Use in Research and Diagnosis, Amer. J. Obstet. Gy ec. 51:316-328, 1946.

45. Roland, Maxwell, The Fern Test, Obstet. Gynec. 11:30-34, 1958.

46. Rubinstein, B.B., The Vaginal Smear--Basal Body Temperature Technic and its Application to the Study of Functional Sterility in Women, Endocrinology 27:843-856, 1940.

47. Rydberg, Erik, Observations on the Crystallization of the Cervical Mucus, Acta. Obstet. Gynec. Scand. 28:172-187, 1948.

48. Sevag, M.G. and Colton, S.W., Simple Chemical Method for the Determination of Ovulation Time in Women, J.A.M.A. 170:13-18, 1959.

49. Siegler, A.M., The Cervical Glucose as an Indicator of Ovulation, Amer. J. Obstet. Gynec. 79:1169-1172, 1960.

50. Siegler, S.Z. and Siegler, A.M., Evaluation of the Basal Body Temperature, Fertil. Steril. 2:287-301, 1951.

51. Speck, George, Elam, James and Palmer, R.E., Colorimetric Test of Sevag and Colton for the Determination of Ovulation, 19:124-125, 1962.

52. Sturgis, S.H., Present Status of Measures for Detecting Ovulation, in Meigs, J.V. and Sturgis, s.H., ed., Progress in Gynecology, Vol. III, New York, Grune & Stratton, 1957, pp. 301-309.

53. Taymor, M.L., Timing of Ovulation by LH Assay, Fertil. Steril. 10:212-226, 1959.

54. Tompkins, Pendleton, The Use of Basal Te perature Graphs in Determining the Date of Ovulation, J.A.M.A. 124:698-700, 1944.

55. Viergiver, Ellenmae and Pommerenke, W.T., Measurement of the Cyclic Variations in the Quantity of Cervical Mucus and its Correlation with Basal Temperature, Amer. J. Obstet. Gynec. 48:321-328, 1944.

56. _____ and _____ Determination of Reducing Substances in Human Cervical Mucus, Amer. J. Obstet. Gynec. 54:459-566, 1947.

57. Wharton, L.R. and Henriksen, Erle, Studies in Ovulation, J.A.M.A. 107:1425-1432, 1936.

58. Wide, Leif, An Innnunological Method for the Assay of Human Chorionic Gonadotrophin, Acta. Endocr. Suppl. 70, 1962.

59. Zondek, Bernhard and Rozin, Samuel, Cervical Mucus Arborization, Obstet. Gynec. 3:463-470, 1954.

60. Zuck, T.T., The Relation of Basal Body Temperature to Fertility and Sterility in Women, Amer. J. Obstet. Gynec. 36:998-1005, 1938.