An article of manufacture and a test is provided for detecting the fertile period or the presence of pregnancy in the female. The article is comprised of a porous support impregnated with at least one color developing compound such as p-nitrophenyl-n-acetyl-β-d-glucosaminide and at least one buffer that develops a color when contacted with a female biological medium. The development of the marked color is coincident with the fertile period, which indication of the fertile period can be used either for achieving fertilization or for preventing conception by the rhythmic method. Also, in the days preceding the expected menstrual period, the development of the marked color is coincident with the presence of pregnancy.

10 Claims, 4 Drawing Figures
FIG. 3

PRIOR ART

% ACTIVITY

DAYS BEFORE MENSTRUATION

FIG. 4

OPTICAL DENSITY

DAYS BEFORE MENSTRUATION
ARTICLE FOR DETECTING THE FERTILE PERIOD AND METHOD FOR USING SAME

BACKGROUND OF THE DISCLOSURE

This invention relates to both a novel article useful for detecting the fertile period of the female, the presence of pregnancy and to a novel test method suitable for detecting the said fertile period or the presence of pregnancy.

In the past, as well as in the present, there has been considerable interest in detecting the fertile period of the female, that is, the time during which the female is capable of reproduction, and also considerable interest in ascertaining the presence of pregnancy. This interest in detecting the fertile period for having an offspring, and also to those who wish to abstain during this period to avoid having an offspring. The interest in detecting the presence of pregnancy is important to those who desire to provide medical care for the health of the female and the offspring and also for interrupting an unwanted pregnancy. However, before this invention, the means and methods used for ascertaining the fertile period or the presence of pregnancy have been fraught with difficulty and uncertainty. For example, one attempt to ascertain the fertile period or the like was based on the idea that the fertile period is usually a certain number of days before menstruation. That is, the period of fertility, or ovulation in the human female is set from 12 to 16 days before the next menstrual period and this calculation is used for the intended purpose. However, calculations based on the onset of mens are often inaccurate because the human female may ovulate at varying and unpredictable times. Additionally, the length of life of the ova and the spermatozoa, which information is not truly known in vivo, must also be added to the calculated period which further decreases the value and reliability of this method. Another method often used for detecting the female period consists in taking basal temperature, but this method is not only inconvenient, it additionally requires considerable skill in observing, recording and interpreting the data. Recently, chemical methods for detecting the fertile period based on an increased monooestrogen or alkaline phosphatase activity were made available to the art. But, while these methods represent an advance in the art, they have the shortcoming of failing to define the fertile period by spreading their results over a prolonged period, or they failed to detect pregnancy. Additionally, other prior art methods had the added shortcoming of lacking a positive ability to effectively ascertain in a seemingly unequivocal manner the presence of pregnancy.

SUMMARY OF THE DISCLOSURE

Accordingly, it is an immediate object of the present invention to provide an article for detecting the fertile period, or the presence of pregnancy that substantially overcomes the difficulties associated with the prior art.

Yet another object of the present invention is to provide a test for detecting the fertile period or the presence of pregnancy which test is convenient, safe, reliable and easy to use.

It is another object of the present invention to provide a simple chemical fertility or pregnancy test which can be carried out by the unskilled layman and which test gives results with accuracies generally better than those obtainable with heretofore known fertility tests.

Yet another primary object of the present invention is to provide a purely chemical or test for ascertaining the period of ovulation, which test does not require animals or complicated calculations, and which test can be simply accomplished in a bath of chemicals with a female biological medium or contacting the medium with a test strip that develops a color to indicate the period of ovulation.

Yet still another object of the present invention is to provide a method for detecting ovulation which detection can be used as a time for achieving conception or as a time for rhythmic contraception.

Yet still another object of the invention is to provide a method for earliest detection of the pregnant state which detection can be used for achieving better medical care.

These and other objects, advantages and features of the present invention will be more apparent from a further reading of the specification and the accompanying claims.

DESCRIPTION OF THE INVENTION

Turning now more detail to the actual and useful test, wherein the test is comprised of a biological female medium such as saliva, vaginal fluid, cervical fluid and the like by mixing an aliquot of the female fluid medium with a color forming reagent and then observing or measuring the developed color. In one procedure, the medium and the reagent are mixed in the presence of a first buffer, and after a short reaction period of about 1 hour, a second buffer is added to more fully intensify the color and the absorbance measured in a standard spectrophotometer. In another test embodiment, a pH indicator is impregnated with a color developing reagent and a buffer and then contacted with a female fluid. After a like short period of time, the test material is dipped or sprayed with a different buffer to more fully develop a visible color. This color test material then is compared against a previously prepared color chart. In both embodiments, the color indicates the fertile period or the presence of pregnancy, and this knowledge can be used for achieving conception or for rhythmic contraception or for ascertaining the pregnant state.

The color developing reagent suitable for the purpose of the present invention is selected from the commercially available compounds p-nitrophenyl- o-acetyl- β- d-glucosaminide, or -n-acetyl- β- d-glucosaminide, l-[p-nitroacetone]-o-acetyl- β- d-glucosaminide, or -n-acetyl- β- d-glucosaminide, 2-[p-nitroacetone]-o-acetyl- β- d-glucosaminide, and 3,3-bis[p-hydroxyphenyl] phthalid-n-acetyl- β- d-glucosaminide. Presently, there is at least one of the above color developing reagents present for the test purpose, although a mixture of these developers can be used.

The buffers used for the purpose of the invention are of two kinds. A first buffer system with a pH range of about 4 to 5, and a second buffer system with a pH range of about 9 to 11. Materials suitable for com-
pounding the first buffer are sodium citrate and citric acid, and the buffer used is 0.1 M adjusted to 10.3.

In one embodiment, the test is conveniently carried out by first mixing a sample of a female fluid, such as, 0.1 ml of saliva with 0.1 ml of buffered substrate comprised of, for example, 0.1 M sodium citrate-citric acid buffer with 0.2 ml of 0.1 M sodium citrate buffer. Then, after shaking until the reagents are mixed, the reagents are permitted to incubate for 30 minutes at room temperature, to allow the enzyme N-acetyl-β-glucosaminidase present in the saliva to hydrolytically act on the substrate p-nitrophenyl-β-acetyl-d-glucosamine. At the end of the incubation period, a second buffer consisting of 1.0 ml of 1.0 M sodium glycinate buffer is added to the reaction to stop the hydrolytic activity and also to develop the color. The absorbance is measured at 400 nm and compared to a standard curve prepared with solutions of known concentrations of p-nitrophenol.

Turning now to the embodiment wherein a bifolous material is impregnated with reagents, generally the bifolous materials suitable for the practice of the invention are those materials, which by means of capillary action or any other physical chemical technique are able to hold liquids. Such materials include test paper that is preferably an absorbant paper, filter paper, cellulose strips, wood strips, felt, porous ceramic strips, assorted cotton substrates and the like. A typical test paper is prepared for oral use or for contacting cervical or vaginal mucous by impregnating the porous strip with, for example, 0.1 to 0.5 ml of buffered substrate consisting of 0.1 M p-nitrophenyl-β-acetyl-d-glucosaminide in 0.1 M sodium citrate buffer. Then, the porous paper is subjected to drying at room temperature, or up to 100°C without changing the paper, to evaporate the aqueous solvent and leave active ingredients on the paper.

The test paper so prepared is used to determine the fertility period as evidenced by changes in, for example, the saliva of the female. In the oral test, the female simply touches the test paper to her tongue to wet it and then waits about 20 to 40 minutes, usually 30 minutes, at room temperature for the increase in N-acetyl-β-glucosaminidase to form a color developing compound. The color is more fully developed by wetting the test strip with a small but effective amount of previously prepared 0.1 M sodium glycinate buffer. The test strip is then compared to a standard color card that has a series of color spots similarly developed from known concentrations of the color developing compound p-nitrophenol. The test implement will change or develop a maximum color during the period of ovulation and fertility of the female, and it will undergo a minimum or no color change at other times. This detection can be used for conception or for rhythmic contraception. The test is used in like fashion for indicating the presence of pregnancy.

The obvious and improved results obtained with the test of this invention are more easily seen in the accompanying Figures. In FIG. 1 there is seen the increased activity of N-acetyl-β-glucosaminidase in saliva during the female menstrual cycle. The results have been normalized by assigning a value of 100 for the highest enzymatic activity during the menstrual cycle. This value always coincides with the peak basal body temperature registered at ovulation. In FIG. 1, the arrow through zero "0" is the beginning of menstruation.

In FIG. 2, the activity of alkaline phosphatase in saliva during the menstrual cycle is presented as measured by a standard prior art method to illustrate the improved and unobvious results obtained by the N-acetyl-β-glucosaminidase method of the invention as seen in FIG. 1. The standard alkaline phosphatase test is performed by first preparing a sodium or potassium glycinate 0.1 M buffer adjusted to pH 9.6 and a buffered substrate comprised of 0.01 M p-nitrophenylphosphate dissolved in glycine buffer, 0.1 M, pH 9.6. The test is performed by adding 1.0 ml of buffered substrate mixture to 0.2 ml of saliva, with shaking and incubation for 45 minutes at 37°C. After this period of time, the color developed is measured at 400 nm, nanometer, and compared to a standard curve. The results in FIG. 2 are presented in a manner identical with the results presented in FIG. 1.

In FIG. 3, the activity of acid phosphatase in saliva during the menstrual cycle is presented as measured by a standard prior art method. This result is presented to illustrate the unobvious and improved results obtained by the inventive glycosidase method as seen in FIG. 1. The standard test is carried out by first preparing an acetic acid sodium acetate 1.0 M buffer adjusted to pH 4.7, a buffered substrate comprised of p-nitrophenylphosphate disodium salt, 0.1 M, dissolved in 0.1 M sodium acetate buffer adjusted to pH 4.7, and lastly an 0.1 M sodium glycinate buffer adjusted to pH 10.3. The test consists of adding 0.1 ml of the buffered substrate and 0.2 ml of acetate buffer to 0.1 ml of saliva, followed by shaking and incubation for 30 minutes at room temperature. At the end of this period, 1.0 ml of glycine buffer is added to the reaction mixture, with the results measured at 400 nm and compared to a standard curve.

FIG. 4 is the determination of the peak, second of maximum activity of N-acetyl-β-glucosaminidase and its relationship with pregnancy. In FIG. 4, the graft is normalized so that the first day of menstruation occurred or was expected to occur. The vertical lined area of the curved area is the range of 40 female patients with normal menstruation. The horizontal lined area of the curved area is the range of pregnancy of eight patients who used the test for achieving this result. The numbers on the left of the Figure represent the optical density, that is, the absorbance or intensity of the color developed for the test.

In summary, the present invention relates to an improved test, an article and method which are especially useful for determining the female fertile period or the presence of pregnancy. The test comprises a color developing compound and buffers, and the test article is a bifolous material impregnated with same; and, while the invention is described in detail, it is understood that the foregoing detailed description is given for the purpose of illustration and that many variations may be made therein without departing from the spirit of the invention.

What is claimed is:

1. A test article useful for detecting an increase of N-acetyl-β-glucosaminidase in a female biological media which is coincident with the onset of the fertile period of a female, which article is comprised of a bifolous material containing impregnated therein a non-toxic buffer capable of maintaining the pH in the range
of 4 to 5 and an effective amount of the color indicator p-nitrophenyl-n-acetyl-β-d-glucosaminide which is present in an increase of N-acetyl-β-glucosaminidase in the female biological media forms a color developing compound useful for detecting the fertile period.

2. A test article useful for detecting an increased N-acetyl-β-glucosaminidase activity according to claim 1 which additionally includes as a color intensifier a separate buffer capable of maintaining the pH in the range of 9 to 11.

3. A test article useful for detecting an increase of N-acetyl-β-glucosaminidase in a female biological media which is coincident with the presence of pregnancy in a female, which article is comprised of a bulbous absorbent strip-shaped material containing impregnated therein a non-toxic buffer capable of maintaining the pH in the range of 4 to 5 and an effective amount of a color indicator selected from the group of indicators consisting essentially of p-nitrophenyl-n-acetyl-β-d-glucosaminide, α-naphthol-n-acetyl-β-d-glucosaminide, 1-[p-nitroacetil]-n-acetyl-β-d-glucosaminide and 3,3-bis[p-hydroxyphenyl]phthalid-n-acetyl-β-d-glucosaminide which indicates in the presence of an increase of N-acetyl-β-glucosaminidase in the female biological media forms a color developing compound wherein the color developed indicates the presence of pregnancy.

4. A diagnostic test for the detection of the fertile period of a female warm-blooded animal which test is comprised of adding a female media selected from the group consisting of saliva, cervical fluid and vaginal fluid to a composition consisting of the color indicator p-nitrophenyl-n-acetyl-β-d-glucosaminide in an amount sufficient to develop a color in the presence of N-acetyl-β-glucosaminidase in the media indicating fertility and ovulation and a biologically acceptable first buffer capable of maintaining the pH in the range of 4 to 5 to form a color developing compound, then adding a second buffer capable of maintaining the pH in the range of 9 to 11 to further develop the color which indicates the presence of said fertile period of the female.

5. A method for indicating when to use rhythmic contraception adapted to a female which method is comprised of determining the fertile period of the female by adding a female media selected from the group consisting of saliva, cervical mucosa and vaginal mucosa to a mixture consisting of the color indicator p-nitrophenyl-n-acetyl-β-d-glucosaminide present in an amount sufficient to develop a color in the presence of N-acetyl-β-glucosaminidase in the media and a biologically acceptable buffer capable of maintaining the pH in the range of 4 to 5 to form a color developing compound, then adding a buffer capable of maintaining the pH in the range of 9 to 11 to further develop the color, which color indicates the fertile period that occurs during ovulation of the female which developed color is a means for determining when to use said method of rhythmic contraception.

6. A method for indicating the fertile period of the female that occurs during ovulation by detecting an increase in the enzyme N-acetyl-β-glucosaminidase which is coincident with the fertile period of the female wherein the method is comprised of contacting female saliva containing the increase of N-acetyl-β-glucosaminidase with the color indicator substrate p-nitrophenyl-n-acetyl-β-d-glucosaminide which is present in an effective amount to form the color developing compound p-nitrophenyl in the presence of a buffer capable of maintaining the pH in the range of 4 to 5, then adding another buffer capable of maintaining the pH in the range of 9 to 11 to further develop the color which color indicates the fertile period of the female.

7. A method for indicating the presence of pregnancy in a female by detecting an increase in the enzyme N-acetyl-β-glucosaminidase which is coincident with pregnancy, wherein the method is comprised of contacting a female fluid excretion with a composition containing the color indicator substrate p-nitrophenyl-n-acetyl-β-d-glucosaminide which is contained in the composition in an effective amount to form the color developing compound p-nitrophenyl in the presence of a buffer capable of maintaining the pH in the range of 4 to 5, then adding a buffer capable of maintaining the pH in the range of 9 to 11 to further develop the color which color indicates the presence of pregnancy in the female.

8. A test article useful for detecting an increase of N-acetyl-β-glucosaminidase in a female biological media which increase is coincident with the onset of the fertile period of a female, which article is comprised of a bulbous material containing impregnated therein a non-toxic buffer capable of maintaining the pH in the range of 4 to 5 and an effective amount of the color indicator α-naphthol-n-acetyl-β-d-glucosaminide which is present in the presence of an increase of N-acetyl-β-glucosaminidase in the female biological media forms a color developing compound useful for detecting the fertile period.

9. A test article useful for detecting an increase of N-acetyl-β-glucosaminidase in a female biological media which increase is coincident with the onset of the fertile period of a female, which article is comprised of a bulbous material containing impregnated therein a non-toxic buffer capable of maintaining the pH in the range of 4 to 5 and an effective amount of the color indicator 1-[p-nitroacetil]-n-acetyl-β-d-glucosaminide which is present in the presence of an increase of N-acetyl-β-glucosaminidase in the female biological media forms a color developing compound useful for detecting the fertile period.

10. A test article useful for detecting an increase of N-acetyl-β-glucosaminidase in a female biological media which increase is coincident with the onset of the fertile period of a female, which article is comprised of a bulbous material containing impregnated therein a non-toxic buffer capable of maintaining the pH in the range of 4 to 5 and the color indicator 3,3-bis[p-hydroxyphenyl]phthalid-n-acetyl-β-d-glucosaminide in an effective amount which is in the presence of an increase of N-acetyl-β-glucosaminidase in the female biological media forms a color developing compound useful for detecting the fertile period.