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Appendix 1

Consent form

Consent form for subject in study of

Antenatal vaginal pH screening for vaginitis

I have been informed that department of Obstetrics and Gynecology, Faculty of Medicine, Khon Kaen University is conducting a study of health of pregnant women. The purpose of the study is look at the prevalence and screening test of vaginitis during pregnancy, Pregnant women who attend antenatal care clinic at Srinagarind hospital during the study period (who have been scientifically selected) are being surveyed.

I agree to participate in this study, understanding that it involves:

1. being interviewed and physical examed include per vaginal examination to collect specimen for microbiological studies for approximately 30 minutes.

2. having my medical records reviewed.

All information will be kept confidential. No one will be identified individually in any publish reports. Only the researchers will have assess to the study.

I understand that my participation is entirely voluntary and that I may refuse to answer any question if I choose, or may withdraw my consent to participate at any time without penalty and without in any way affecting the health care I receive. I understand that there are no special risks involve in being a subject and that, eventhough I will not benefit individually, it is expected that other women will benefit from the knowledge gained from the study.

I have been given an opportunity to ask questions about the study and if I have further question about this study, I may contact the investigator(s) in this hospital.

....

Subject's signature Physician's signature

Appendix 2

Screening form for subject selection

Subject selection form in study of

Antenatal vaginal pH screening for vaginitis

	yes	no
1. Sexual intercourse in the previous 24 hours.	[ ]	[ ]
2. Vaginal douche in the previous 24 hours.	[ ]	[ ]
3. Pregnancy complication :	[ ]	[ ]
* abortion or		
* preterm or		
* rupture amniotic membrane or		
* antepartum hemorrhage or		
* placenta previa or		
* cervical incompetence.		
4. Antibiotic use in the previous 7 days.	[ ]	[ ]
5. Per vaginal examination in the previous 24 hours	[ ]	[ ]
6. In labour.	[ ]	[ ]

If the answer to any of the above question is/are "yes", this case should not be recruited into the study.

Appendix 3

Data collecting form

Data collecting form for research topic of

Antenatal vaginal pH screening for vaginitis

For Master Degree, Faculty of Medicine, Chulalongkorn University

****

I. Identification and demographic data

1. study number..... [ ][ ]

2. subject's name.....

3. subject's address.....

.....

4. hospital number..... [ ][ ][ ][ ][ ][ ]

5. date of examination..... [ ][ ][ ][ ][ ][ ]

II. General information

6. birth date..... [ ][ ][ ][ ][ ][ ]

7. age.....years [ ][ ]

8. race [ ]

1. Thai
2. Chinese
3. Indian
4. Other, specify.....

9. religion [ ]

1. Buddhist
2. Christian
3. Muslim
4. other, specify.....

10. education [ ]

0. never learn in school
1. primary school
2. secondary school
3. High school
4. graduated
5. other, specify.....

11. occupation [ ]

1. farmer
2. trader
3. housewife
4. labourer
5. government service
6. general employee
7. other, specify.....
9. unknown

12. husband's occupation [ ]

1. farmer
2. trader
3. labourer
4. government service
5. general employee
6. other, specify.....
9. unknown

13. subject's weight.....kilograms [ ][ ]

14. subject's height.....centimeters [ ][ ][ ]

III. Obstetrical & gynecological history

15. age of menarche.....years [ ][ ]

16. gravidity (total number of pregnancy)..... [ ][ ]

17. parity (total number of delivery)..... [ ][ ]

18. outcome of previous pregnancy

	number of pregnancy				
	1	2	3	4	5
18.1 live birth	[ ]	[ ]	[ ]	[ ]	[ ]
18.2 stillbirth	[ ]	[ ]	[ ]	[ ]	[ ]
18.3 spontaneous abortion	[ ]	[ ]	[ ]	[ ]	[ ]
18.4 Induced abortion	[ ]	[ ]	[ ]	[ ]	[ ]
18.5 abortion	[ ]	[ ]	[ ]	[ ]	[ ]
18.6 ectopic pregnancy	[ ]	[ ]	[ ]	[ ]	[ ]
18.7 molar pregnancy	[ ]	[ ]	[ ]	[ ]	[ ]
18.8 preterm labour & term delivery	[ ]	[ ]	[ ]	[ ]	[ ]
18.9 preterm delivery	[ ]	[ ]	[ ]	[ ]	[ ]
18.10 PROM	[ ]	[ ]	[ ]	[ ]	[ ]
18.11 PPROM	[ ]	[ ]	[ ]	[ ]	[ ]
18.12 unknown	[ ]	[ ]	[ ]	[ ]	[ ]

19. previous experience of vaginal infection [ ]

0. no

1. yes, specify.....

19.1 treatment by [ ]

0. none

1. doctor's prescription

2. other, specify.....

19.2 duration of treatment [ ]

1. a few days

2. a few weeks

3. a few months

4. more than 3 months, specify.....

20. previous experience of STD [ ]

0. no

1. yes, specify.....

20.1 treatment by [ ]

0. none

1. doctor's prescription

2. other, specify.....

20.2 duration of treatment [ ]

1. a few days

2. a few weeks

3. a few months

4. more than 3 months, specify.....

21. husband's STD history [ ]

0. no

1. yes, specify.....

9. unknown

21.1 treatment by [ ]

0. none

1. doctor's prescription

2. other, specify.....

21.2 duration of treatment [ ]

1. a few days

2. a few weeks

3. a few months

4. more than 3 months, specify.....

IV. Current pregnancy

22. order of this pregnancy..... [ ]

23. LMP (last menstrual period)..... [ ][ ][ ][ ][ ][ ]

24. gestational age.....weeks [ ][ ]

25. last number of ANC attend ..... [ ][ ]

26. complaint of any obstetrical or gynecological problems [ ]

0. no

1. yes, specify.....

V. Personal characteristic

27. smoking [ ]

0. no

1. ex-smoker

2. current smoking

28. alcohol drinking [ ]

0. no

1. ex-drinker

2. current drinking

VI. Per vaginal examination

29. external genitalia apperance [ ]

1. normal

2. abnormal, specify.....

30. vagina (mucosa, discharge) [ ]

1. normal

2. abnormal, specify.....

31. cervix [ ]

1. normal

2. abnormal, specify.....

32. uterus [ ]

1. normal, size correlate with gestational age

2. abnormal, specify.....

33. adnexa [ ]

1. normal

2. abnormal, specify.....

34. cul-de-sac [ ]

1. normal

2. abnormal, specify.....

VII. Microbiological studies

35. Papanicolaou smear cytological number..... [ ][ ][ ][ ][ ][ ]

35.1 Papanicolaou smear result (class 1-5)..... [ ]

description.....

.....

36. culture for G.C., GBS., fungus [ ]

0. negative

1. positive

37. ELISA for chlamydia [ ]

0. negative

1. positive

38. gram stain [ ]

0. negative

1. positive, specify.....

39. Amine odour (Whiff test) [ ]

0. negative

1. positive

40. wet mount examination [ ]

0. negative for pathogenic organism

1. positive clue cells

2. positive fungus

3. positive T.V.

4. positive G.C.

5. other, specify.....

41. pH level by pH paper (range 3-8)..... [ ]

Appendix 4

Host Response for Specific Vaginal Pathogens

In bacterial vaginosis: Facultative lactobacilli maintain the acid pH of the vagina by the metabolism of glucose generated by glycogenolysis. Low pH directly inhibits the growth of some organisms, including anaerobic organisms, by maintaining a higher oxidation-reduction potential. Hydrogen peroxide produced by facultative lactobacilli also might control the growth of catalase-negative organisms like anaerobes. In patients with BV, lactobacilli are replaced by G. vaginalis and a mixed predominantly anaerobic flora. The number of anaerobic organisms that were greatly increased in BV included *Bacteroides bivius*, *B. disiens, B. melaninogenicus*, peptostreptococci, peptococci, and *Eubacterium species*. BV is a poly microbial condition in which a decrease in vaginal acidity and in the concentration of lactobacilli is accompanied by an increase of a 100-fold or more in the concentration of other organisms.

In Trichomoniasis: The vaginal pH is elevated above 4.5 in as many as 90% of cases. This findings, however is nonspecific as 90% of women with bacterial vaginosis will also have an elevated pH. A helpful test in differentiating trichomonas from bacterial vaginosis is elevation of the wet smear for polymorphonuclear cells (PMNs) and "clue cells". The ratio of PMNs to vaginal epithelial cells is greater than 1 in 75% of trichomonal infection, where as in bacterial vaginosis this ratio is less than 1 in 90% of patients. Also, vaginal epithelial cells appear normal in trichomonal

infection, whereas they have the characteristic ill-defined border and bacterial stippling (clue cells) in bacterial vaginosis.

In chlamydial infection, the initiation of infection depends on what appears to be specific attachment sites on susceptible host cells. The initial step in this process is attachment of the metabolically inactive but infectious elementary body to a susceptible host cell. The host cells are generally nonciliated columnar or cuboidal epithelia such as those found in the conjunctiva, urethra, endocervix and the mucosa of the endometrium and fallopian tubes. After the elementary body attaches to the host cell, it is ingested by a phagocytic process which is similar to ordinary bacterial phagocytosis. The process is an enhanced phagocytosis, which is induced by the elementary body, which then are selectively taken up by the susceptible host cell. Intracellular, the elementary bodies exist within a cytoplasmic vacuole. Chlamydiae remain within this phagosome throughout their entire growth cycle. In this state, chlamydiae may be protected from host defense mechanisms such as cellular lysosomes. Such protection perhaps is responsible for the chronicity of certain chlamydial infections and do not change vaginal pH level. In the next step of the chlamydial growth cycle, the elementary body undergoes reorganization into what is called a reticulae or initial body, which represents the metabolically active and dividing form of the organisms. These forms are not infectious and will not survive outside the cell. They divide for approximately 8-24 hr. and then condense and reorganize to form new elementary bodies. For their entire intracellular life, the chlamydiae reside within the phagosome, but they successfully prevent phagolysosomal fusion. Recognition of the characteristic cytoplasmic inclusion is the

means by which chlamydiae are detected. Infectivity increases as the number of elementary bodies increases, and by 48-72 hr, the host cell bursts and liberates these infectious particles or the inclusion is extruded intact by a process of reverse endocytosis. The cycle then starts a new. The complete infectious cycle takes 2-3 days[7].

For candidiasis inflammatory process, it gain access to the vaginal lumen and secretion predominantly from the adjacent perineal area. t was conclude that *C. albicans* was never a commensal in the vagina and was always a pathogen in one study[7], and clinicians usually can detect vaginal pathologic findings even in asymptomatic patients from whom candida species were isolated. Subsequent investigators, however not corroborated this view, demonstrating that many women carry *C. albicans*, usually in low numbers, in their vaginas but have no symptoms. These observations are compatible with the view of *C.albicans* as a vaginal commensal and pathogen, indicating that changes in the host vaginal environment or response usually are necessary before candida induces the pathologic effects or is associated with symptoms. Candida-associated vaginitis is seen predominantly in women of child-bearing age only in minority of cases can a precipitating factor be identified to explain the transformation from asymptomatic carriage to symptomatic vaginitis in individual patients[35].

Candidal organisms are dimorphic fungi, i.e., they may be found in humans in different phases. In general, blastospores represent the phenotypic form responsible for transmission or spread, and they are the form associated with a symptomatic vaginal colonization. By contrast germinated yeast that are producing mycelia most

commonly constitute the tissue invasive form and are usually identified in association with symptomatic disease[36]. During the symptomatic episode there is the conspicuous appearance of the germinated or filamentous forms of candida. Germinated organisms not only enhance colonization but represent the dominant invasive phase capable of penetrating intact epithelial cells and invading the vaginal epithelium, although only the very superficial layers are involved. Although symptoms are not strictly related to the yeast load, nevertheless clinical vaginitis does tend to be associated with greater number of organisms and with the germinated yeast phase. Approximately 10^3 - 10^4 candida per ml. of vaginal fluid may be recovered in both symptomatic and asymptomatic states.

The mechanism whereby candida induces inflammation is not yet established. Yeast cells are capable of producing several extracellular protease as well as phospholipase. The paucity of phagocytic cells in the inflammatory exudate possibly reflects the lack of chemotactic substances elaborated. Both blastoconidia and pseudohyphae are capable of destroying superficial cells by direct invasion.

Based on the clinical spectrum which varies from an acute florid exudative form with thick white vaginal discharge and large number of germinated yeast cells to the other extreme of absent or minimal discharge, fewer organisms, and yet severe pruritus, it is suggested that more than one pathogenic mechanism may exist. In the presence of pruritus alone, hypersensitivity or immune mechanisms are likely to be involved of asymptomatic female carriers of candida develop postcoital penile erythema and pruritus which usually lasts several hours only[26].

To diagnosis candidal vaginitis, most patients with symptomatic vaginitis may be readily diagnosed on the basis of simple microscopic examination of vaginal secretions. Accordingly, a wet mount or saline preparation should routinely be done, not only to identify the presence of yeast cells and mycelia but also to exclude the presence of "clue cells" and motile trichomonads. Large number of white cells are also invariably absent and when present should suggest a mixed infection. The 10% KOH preparation is extremely valuable and even more sensitive in diagnosing the presence of the germinated yeast. Similarly, vaginal pH estimations reveal a normal pH (4.0-4.5) in candidal vaginitis, and the finding of a vaginal pH in excess of 5.0 should strongly indicate mixed infection.

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VITAE

Jadsada Anansuwanchai was born on the 27th June 1962 at Khon Kaen Province, Thailand. Graduate degree of Bachelor of medicine from Faculty of Medicine, Khon Kaen University in 1987. Graduate Thai Board of Obstetrics and Gynecology in 1991. Then working as the staff of Department of Obstetrics and Gynecology, Faculty of Medicine, Khon Kaen University.

Jadsada Anansuwanchai studied in the course of Clinical Epidemiology of Thai-CERTC at Chulalongkorn University since June 1993 funding by the Rockefeller Foundation and expect to receive master degree in 1995. At present working as the staff of Department of Obstetrics and Gynecology, Faculty of Medicine, Khon Kaen University.