

VAGINAL SMEAR - FERN TEST **pada Kucing dan Domba^{*)}**

M.K. Ilmu Kebidanan dan Kemajiran

Div. Reproduksi dan Kebidanan

Dept Klinik Reproduksi dan Patologi

Fakultas Kedokteran Hewan IPB



Vaginal smear cytological examination

Haralabos Ververidis
DVM, PhD, Associate Professor
Companion Animal Clinic, Faculty
of Veterinary Medicine A.U.Th.

Constantinos Boscas
DVM, PhD, Professor
Farm Animal Clinic, Faculty of
Veterinary Medicine A.U.Th.

Obtaining the specimen



Figure 1. Drawing of a sagittally incised vagina. The sampling site for vaginal cytological examination is marked red.



Figure 2. Equipment needed to obtain a vaginal smear. Vaginal speculum, cotton-tipped swab, normal saline solution in a syringe, glass slides for microscopy.



Figure 3. Single use syringes with their front end carefully cut, modified as vaginoscopes that allow the passage of the cotton tipped swab to obtain the vaginal smear. The 1 ml syringe is very suitable for small sized bitches.



Figure 4. External genital organs of the bitch. The dorsal vulvar commissure, the vestibule, the clitoral fold and fossa are (downwards) distinguished.



Figure 5. Preparation of the cotton tipped swab, soaked with 1-3 tiny drops of normal saline solution.



Figure 6. The vulva is partly opened by slight traction of the left labium.
Figure 7. The tip of the vaginal speculum pulls the dorsal commissure backwards and enters into the vestibule with upward direction. When the vagina is relatively dry it is suggested to soak the tip of the speculum with normal saline solution.

Figure 8. The tip of the speculum is introduced with upward direction keeping in touch with the dorsal wall of the vestibule, to avoid the tip from entering the clitoral fossa which would cause reaction of the bitch (lordosis etc.).

Figure 9. When the tip of the speculum has overcome the ischiatic arch (under the level of the anus) it is directed forward and inserts into the horizontal part of the vagina. The left labium is kept slightly tracked to help slipping of

the speculum, while preventing the labia and hair introversion.

Figures 10 & 11. While the speculum tip is kept pushed in the vagina the speculum handle is turned downwards 180°. This movement will facilitate sample collection by the wider dilation of the dorsal part of the vagina.

Figure 12. The speculum handle is transferred to the left hand. Now the right hand is free to collect the sample.



Figure 13. The cotton tipped swab is held standby by the right hand. The handle is pressed so that an adequate vaginal dilation is achieved. Then the cotton tipped swab is inserted, without touching the hair or the vulva. The swab touches the dorsal vagina, where from the vaginal epithelial cell sample is collected by gentle contact - rotational movement (see also figure 1). **Figures 14 & 15.** After the swab withdrawal, the speculum is withdrawn, partly dilated to avoid capture of the vaginal folds, by reversion of the previous movements. **Figure 16.** Vaginal sample for smear preparation may be collected through a makeshift vaginoscope (see also figure 3).

Fixation, staining and observation

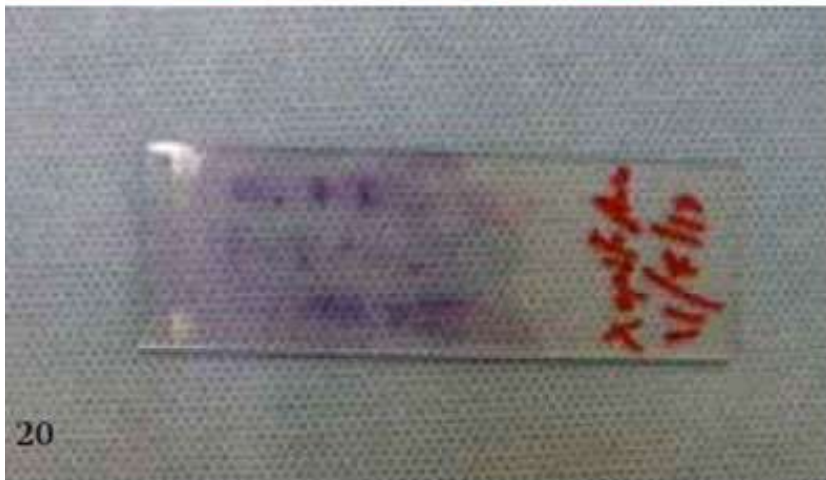


Figure 17. Smear preparation by rolling the swab vertically on a clean glass slide.

Figures 18 & 19. Commercially available ready to use quick staining solutions, suitable also for vaginal smears. Giemsa stock solution before dilution.

Figure 20. Final smear stained, ready for microscopy. Three lines with adequate, properly stained cell material and the marking on the slide can be seen.

MAIN VAGINAL EPITHELIAL CELL TYPES

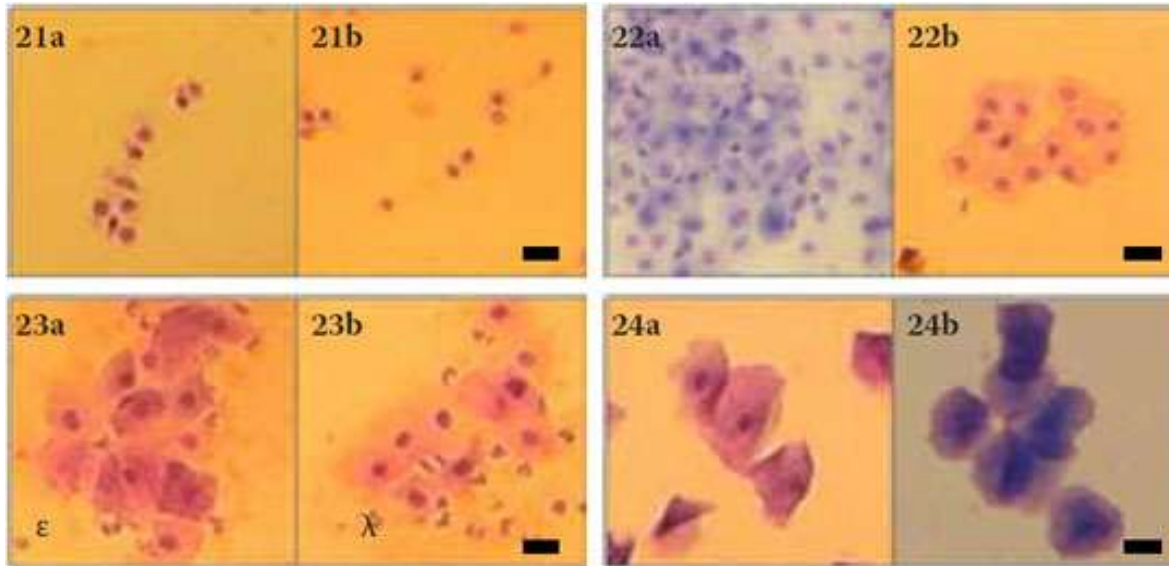


Figure 21 (a & b). Parabasal cells (x400, bar length: ~20 μm).

Figure 22 (a & b). Small intermediate cells (x400, bar: ~20 μm).

Figure 23 (a & b). Large intermediate cells, erythrocyte (ϵ) and neutrophils (λ) (x400, bar: ~20 μm).

Figure 24 (a & b). a. Nucleated – partly keratinized cells, b. fully keratinized cells (x400, bar: ~20 μm).

CYTOLOGICAL PATTERNS DURING THE STAGES OF THE OVARIAN CYCLE

ANESTRUS vs PROESTRUS

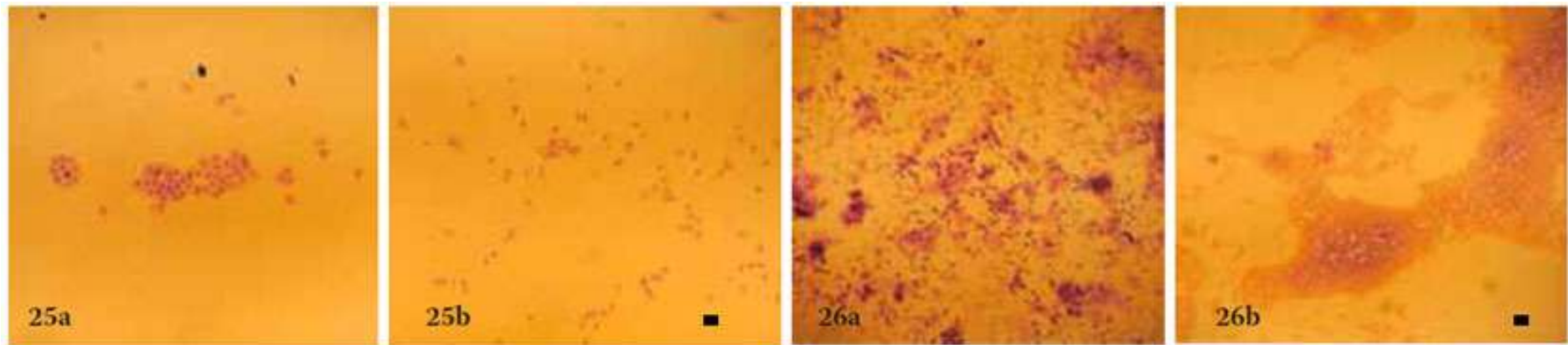


Figure 25a. Anoestrus: small intermediate cells 90%, parabasal cells 10%. **Figure 25b.** Anoestrus: parabasal cells 85%, small intermediate cells 5%, nuclei of ruptured cells 10% (x100, bar: ~20 μ m).

Figure 26a. Proestrus: large intermediate cells 70%, small intermediate cells 20%, keratinized cells 10% and many erythrocytes (++) . **Figure 26b.** Proestrus: large intermediate cells 100% and accumulations of erythrocytes (+++) (x100, bar: ~20 μ m).

Small Intermediate 90%

Parabasal 10%

Small Intermediate 5%

Parabasal 85%

Large Intermediate 70%

Small Intermediate 20%

Keratinized 10%

RBC

CYTOLOGICAL PATTERNS DURING THE STAGES OF THE OVARIAN CYCLE

ESTRUS vs DIESTRUS

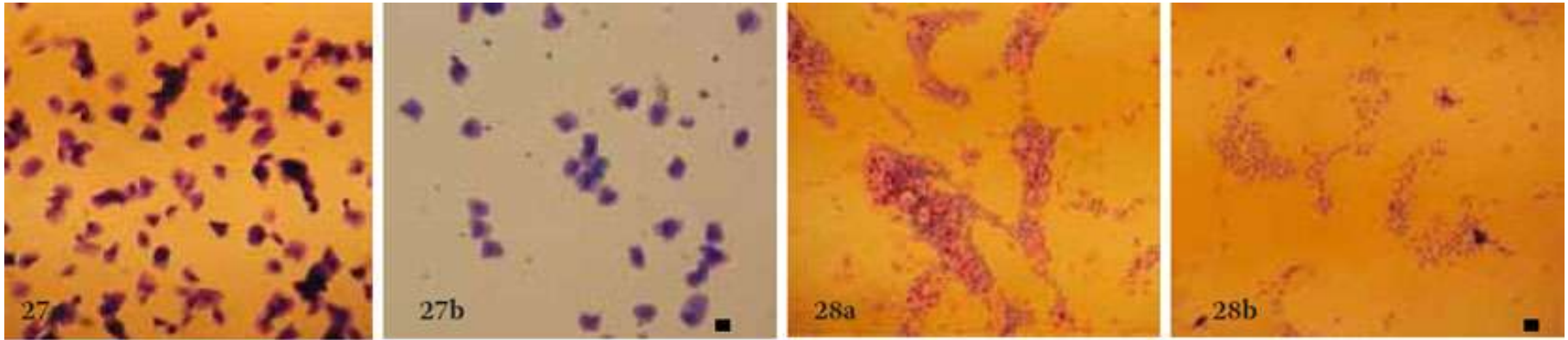


Figure 27a. Oestrus: keratinized cells, partly 50% and fully 50%.
Figure 27b. Oestrus: keratinized cells, fully 70% and partly 30%
 (x100, bar: ~20 µm).

Figure 28a. Early dioestrus: large intermediate cells 40%, small intermediate cells 60% and many mature neutrophils (+++).
Figure 28b. Advanced dioestrus: small intermediate cells 40%, parabasal cells 60% and very few neutrophils (±). The pattern is almost the same as that of anoestrus (x100, bar: ~20 µm).

Keratinized:

Partial 50 – fully 50%
 Partial 30 – fully 70

Early:

Large Intermed 40%
 Small Intermed 60%
 NET +++

Advanced:

Small Intermed 40%
 Parabasal 60%
 NET +/-

Ferning Test

Fern test

- Fern test refers to visualization of a characteristic 'fern-like' pattern on a slide (pre-cleaned, saline free slides are required), viewed under low power on a microscope
- A small amount of cervical mucus is allowed to air-dry on a clean, saline-free glass slide

Procedure:

1. When the slide has completely air dried (at least 5 to 7 minutes), place it on the stage of the light microscope provided for the procedure.
2. Examine the slide under low power (10X).
3. Look for fern-like crystals. If positive for amniotic fluid, this crystal formation will be present in most microscopic fields.

Ovulation Period

Saliva outline

(Drop of saliva outline)

Saliva Water

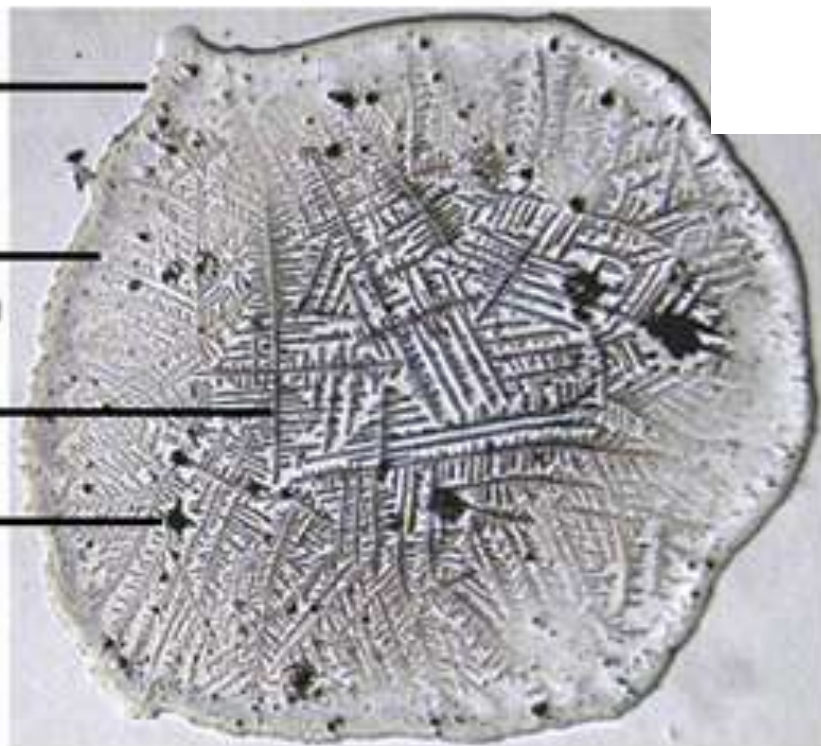
(saliva is 98% water - Hard to see)

Fern Patterns

(Crystals)

Salivary Cells

(Electrolytes, mucus, enzymes)



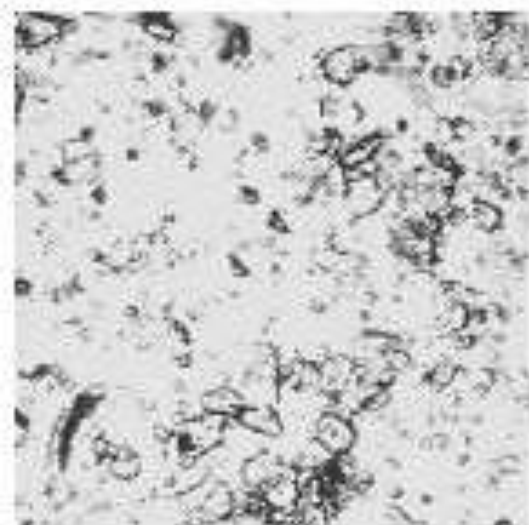
Reading Results

A crystal or - "ferning" - pattern indicates a positive result for ovulation - your most fertile time.

Fertile!

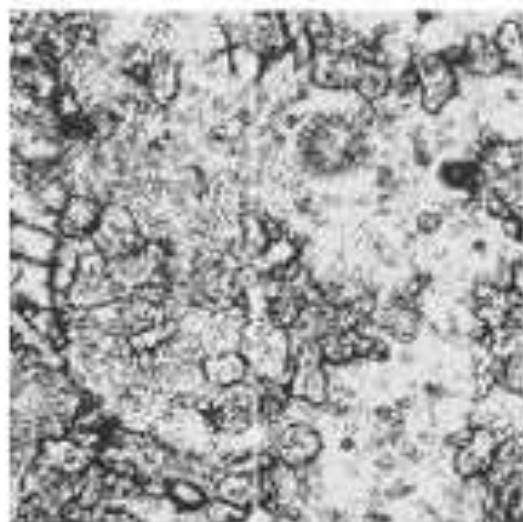


When ovulating the saliva sample should be almost completely covered by ferns/crystals.



Non-fertile

Only dots and some lines appear



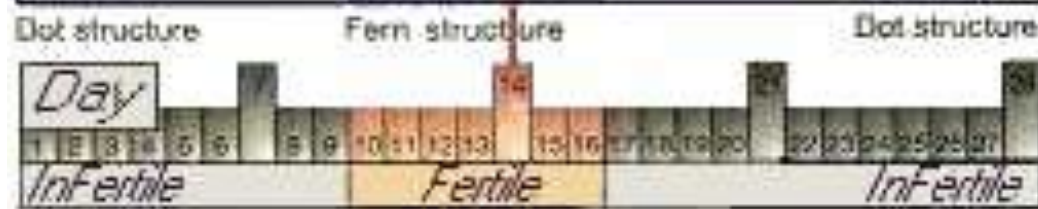
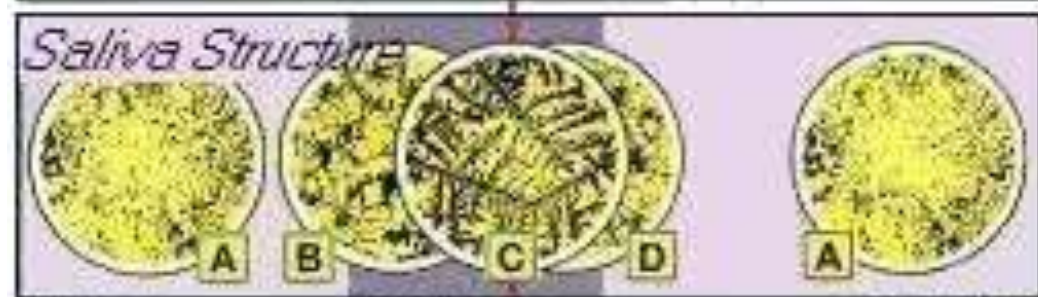
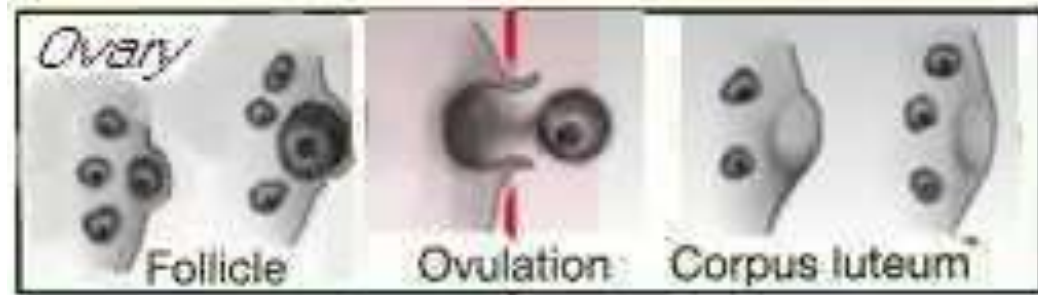
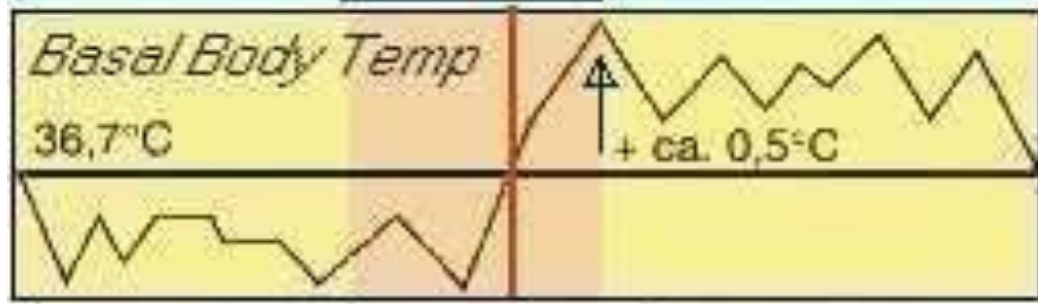
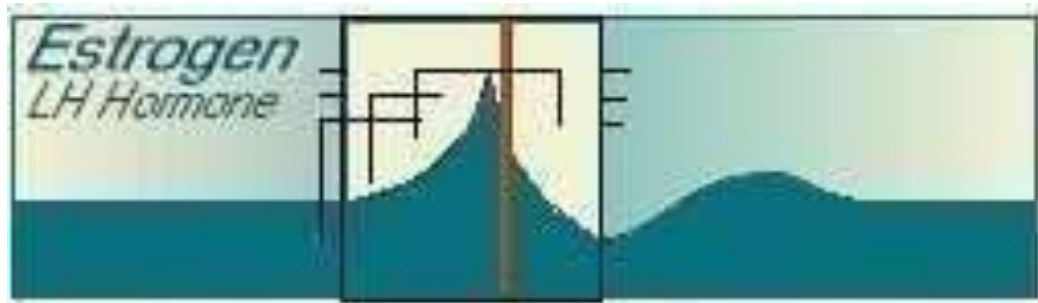
Transitional

Some fern patterns start to appear












Fertile

A lot of ferning patterns appear



Saliva Crystallization in Bitch

"TYPICAL" HEAT CYCLE FOR A DOG - Every dog is different and the coloured areas may be shorter or longer than shown. *Fertile-Focus Ovulation Microscope*® WWW.LAPDOG.CO.NZ Ph 0800 LAPDOG

Cycle Day	8	9	10	11	12	13	14	15	16	17	
Positive Ferning Result											
Standing Heat			X	X	X	X	X	X	X		
Ovulation			LH Surge	←	X	→					
Eggs Sperm Friendly (Follides rupture within 12 to 96 hours - all eggs are not released at the same time, but are in similar stages of development)							←	X	X	X	→
Mating Pattern #1 for fresh semen								X	X	X	
Mating Pattern #2 for fresh semen					X			X		X	
Approx. Progesterone using Immulite analysis method				<5	5	7.5	10	15	18	20	

Note: some bitches rise quickly and ovulate 24 hours after the start of the LH surge and some take longer and ovulate 48 to 72 hrs after the surge of the LH surge.

The ripening of the eggs is 12 to 96 hours after ovulation. This is when the eggs become sperm friendly.

Note: the ovulation and egg process may only last as long as 24 to 36 hours in some bitches. Normally it will last 48 - 96 hours.

Saliva Crystallization in Orangutans

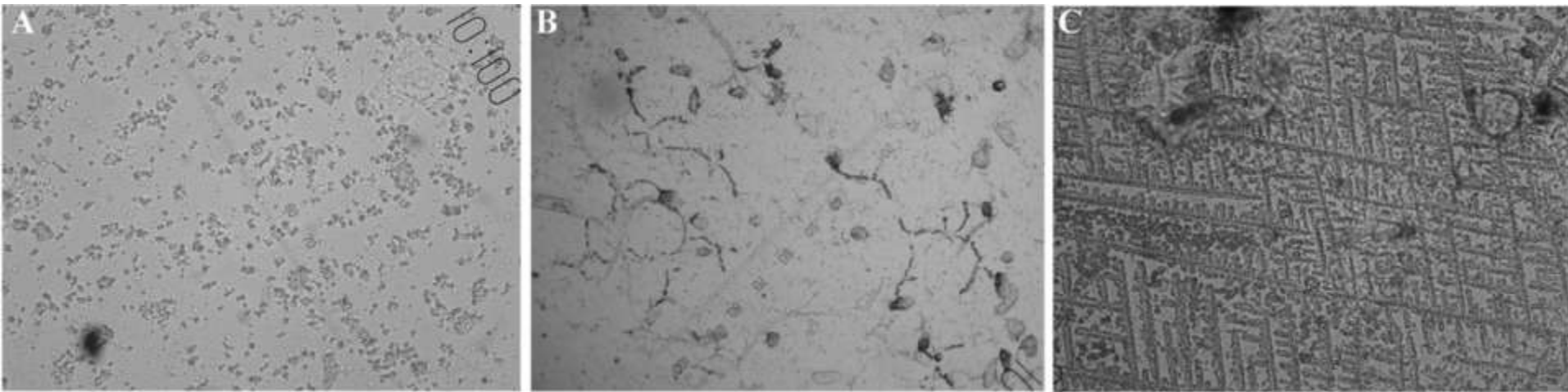


Fig 1. Crystallization typical for non-fertile days (A), transit period (B) and fertile days (C) ($\times 400$).

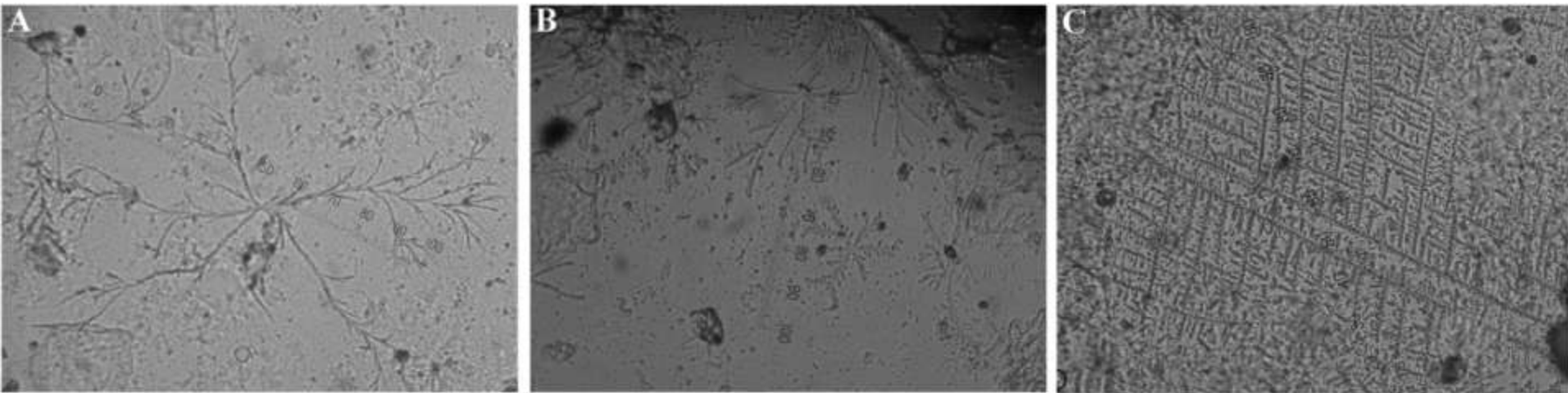


Fig 2. Branch-like (A), fir-like (B) and fern-like (C) crystallization ($\times 400$ magnification).



Tarik, bukan putar



Oleskan air liur pertama di pagi hari pada lensa mikroskop (jangan terbalik)



Tunggu 5 menit sampai kering. Pasang lagi mikroskop pada bodi



Keker mikroskop sambil tekan lampu dibelakang. (zoom dengan memutar lensa mikroskop)

Manual Use of Scope

1. https://www.youtube.com/watch?v=D1pOzo_kaTI
2. <https://www.youtube.com/watch?v=XiB7Z1EwLZk>
3. <https://www.youtube.com/watch?v=8AyywdCFXpU>