Module 3 – Pap Test Protocol

Adapted from various sources by Nancy Pelaez, Onyx Uzomah and Hector Zumbado-Ulate, Sept. 25, 2015.

Objectives

Be able to:
1. Use swabbing technique and cell fixation to determine whether myometrium tissue was isolated from a pre-estrus or post-estrus rat based on Gill's Modified Papanicolaou Stains.
2. Interpret vaginal smears with an adaptation of the Papanicolaou (PAP) procedure for assessing stages of the rat estrus cycle.

Background

Introduction

• The Papanicolaou (PAP) stain was developed by Dr. George N. Papanicolaou. It uses stains and counterstains where tissue is overstained followed by removal of excess stain.
• The oxidation product of hematoxylin is hematin. Hematin exhibits indicator-like properties, being blue and less soluble in aqueous alkaline conditions, and red and more soluble in alcoholic acidic conditions.
• The stain is applied longer than necessary, resulting in the overstaining of the tissues with much non-specific background coloration.
• Background color is selectively removed by leaching in alcohol.
• Returning to an alkaline environment, hematin takes on a blue hue, the process of "blueing-up." The hematin colors cell nuclei blue.

The Papanicolaou (PAP) stain: Eosin

• Cellular detail is obtained by counterstaining the cytoplasm with the eosin, an alcohol-based counter-stain that gives cytoplasm a contrasting color (pink)
• The mechanism of staining is not fully understood, but is believed to be of an electrostatic nature.
• More intense specific coloration is obtained by applying the dyes in acidic conditions, with the more acidic and metabolically active tissue components taking up the dye to a greater intensity.

The Papanicolaou (PAP) stain: Orange G

• Another counterstain uses the alcohol based Orange G stain before the eosin staining procedure.
• The use of Orange G in the Papanicolaou stain is to stain acidic proteins like keratin.
• Its color is orange in neutral and acidic pH or red in pH greater than 9.

METHODS

Materials

• Swabs (soaked in 1% sodium citrate solution or in 1/4-strength Ringer solution, releasing cells for greater recovery)
• MOPS or Kreb’s PSS (to moisten the swabs)
• Pap Smear collection slides & spray Fixative
• Hematoxylin Stain Solution, Gill 2 Formulation
• Scott’s Tap water Substitute, Bluing Agent - a gentle bluing solution (aqueous sodium bicarbonate & magnesium sulphate solution)
• Papanicolaou Stain, Gill’s Modified OG-6 Formula, Counterstain 1
• Papanicolaou Stain, EA-36-50 Formula, Counterstain 2
• CitriSolv® Hybrid Solvent and Clearing Agent - A solvent mixture of hydrocarbon material and limonene derived from citrus for drying
Procedure

Estrous cycle progression can be determined by vaginal smear and PAP staining done with variations according to the protocol described in Hubscher, Brooks, and Johnson (2005). In summary, rats are swabbed once a day (between 11AM and 2 PM) as follows: A sterile cotton swab moistened with PSS or Krebs’s solution is gently inserted approximately 3-5mm into the rat’s vagina to collect cells. The cells are then rolled onto a slide carefully labeled at the frosted end. The cells are sprayed with fixative and allowed to dry. The slides are then stained according to the adapted PAP stain protocol with the steps described below. After the final step, the slides are allowed to air-dry in a dark drawer and then examined under a light microscope at 100X total magnification.

1. 95% ethanol 5 min. and repeat with fresh alcohol once.
2. Tap water 10 quick dips and repeat with fresh water once Gill’s Hematoxylin 2 min.
3. Tap water 10 quick dips and repeat with fresh water once
4. Scott’s tap water substitute 1 min.
5. Tap water 10 quick dips and repeat with fresh water once \* Orange G6 1 min.
6. 95% ethanol 10 quick dips and repeat with fresh alcohol once
7. 95% ethanol 10 quick dips and repeat with fresh alcohol once
8. 95% ethanol 10 quick dips and repeat with fresh alcohol once
9. Eosin-azure 50 10 min. 0
10. 95% ethanol 20 quick dips and repeat with fresh alcohol twice
11. 100% ethanol 10 quick dips and repeat with fresh alcohol twice
12. CitriSolv* Hybrid Solvent and Clearing Agent 10 quick dips and repeat with fresh Solvent twice
13. Air dry and store slides in a dark drawer.

DISCUSSION QUESTIONS

1. What does it mean when people say that the typical rat estrous cycle takes four to five days?

2. The diagram above (from Hubscher, Brooks, & Johnson, 2005) suggests criteria to identify sequential points in the rat estrus estrous cycle from PAP stained vaginal smears. Based on what you saw in the Pap smear sequence for one single rat for at least 5 consecutive days, what do you think Nu, Lk, and Cn mean?

3. According to the evidence collected from our rats, were their estrous cycles in sync or not?

4. Why does the doctor who performs a Pap test on a woman ask the date of her last period?
Source Materials

  http://www.tandfonline.com/doi/full/10.1080/10520290500138422

  http://onlinelibrary.wiley.com/doi/10.1002/0471142301.nsa04is48/full

  https://books.google.com/books?id=vnRpW-gi9JMC&lpg=PP1&pg=PA98#v=onepage&q&f=false and  
  https://books.google.com/books?id=vnRpW-gi9JMC&lpg=PP1&pg=PA100

- Gill's Modified Papanicolaou Stains  