Use of vaginal impedance to stage the estrous cycle in rats given Luteinizing Hormone Releasing Hormone

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Background

Vaginal cytology is the gold standard for staging estrus in female rats; however, it:

- Requires extra training, is time consuming, and is subject to interpretation

Vaginal impedance has been used successfully in normally cycling and breeding female rats to determine proestrus (PE)

- Measures fluctuations in the inherent electrical resistance of the inner lining of the vaginal wall
- Quicker and less subjective compared to vaginal cytology

Estrous cycle monitoring is rarely utilized for female rats undergoing estrus synchronization due to the difficulties of vaginal cytology, therefore success using vaginal impedance could potentially increase breeding efficiency

Objective

To determine whether vaginal impedance measurements can accurately predict proestrus in female rats primed with luteinizing hormone releasing hormone (LHRH) for estrus synchronization.

Materials and Methods

Day -4 — 9am — Intraperitoneal injection of LHRH

Day -1 — 1:30pm — Vaginal impedance measurement and vaginal cytology

Day 0 — 1pm — Pair with proven male breeders

Day 1 — 8am — Vaginal plug check and vaginal cytology for sperm

Day 10+ — Post-mortem assessment of pregnancy

Three (3) groups of 12 female Sprague Dawley (Crl:SD) rats were treated and evaluated according to the procedure and timeline above. Vaginal impedance was measured using the MK-11 Impedance Checker (Muromachi Kikai Co., Ltd., Tokyo, Japan). Measurements greater than 3 kΩ on Day -1 were indicative of poestrus. Vaginal cytology was performed using a blunted glass pasteur pipet and phosphate-buffered saline (PBS). The slides were allowed to air dry before staining with Diff-Quik (Siemens Healthcare Diagnostics, Inc., Deerfield, IL).

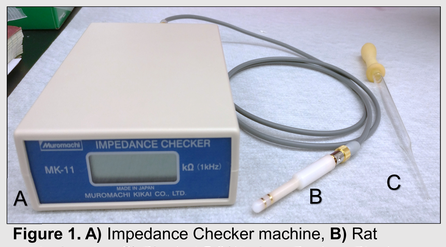

Figure 1. A) Impedance Checker machine, **B)** Rat vaginal probe, **C)** Blunted 5 3/4" glass Pasteur Pipet with bulb

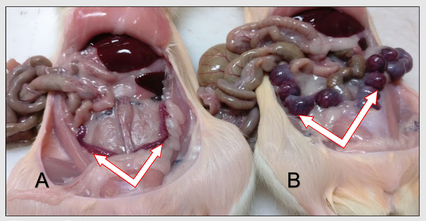

Figure 2. Comparison between pregnant and non-pregnant rats on post-mortem at day 13 post-mating. **A)** Arrows indicate normal, non-pregnant uterine horns. **B)** Arrows indicate implanted embryos in uterine horns.

Table 1. Overall results

Group	Rats (n)	Impedance > 3 kΩ	PE by Vaginal Cytology	Total Positive for Presence of Sperm	Total Pregnant
1	12	6	5	8	5
2	12	7	7	7	5
3	12	5	6	8	9
Total	36	18	18	23	19

Results

Table 2. Direct comparison between impedance value and vaginal cytology

		Vaginal Cytology	
		PE	Not PE
Vaginal Impedance	> 3 kΩ	14	4
	< 3 kΩ	4	14

Table 3. Comparison between pregnancy status and impedance value and vaginal cytology

	Pregnant	Not Pregnant
> 3 kΩ by Impedance	8	10
< 3 kΩ by Impedance	11	7
PE by Cytology	8	10
Not PE by Cytology	11	7

Table 4. False positive and false negative rates related to pregnancy status

Impedance	False Positive	10/36 = **28%**
	False Negative	11/36 = **31%**
Cytology	False Positive	10/36 = **28%**
	False Negative	11/36 = **31%**

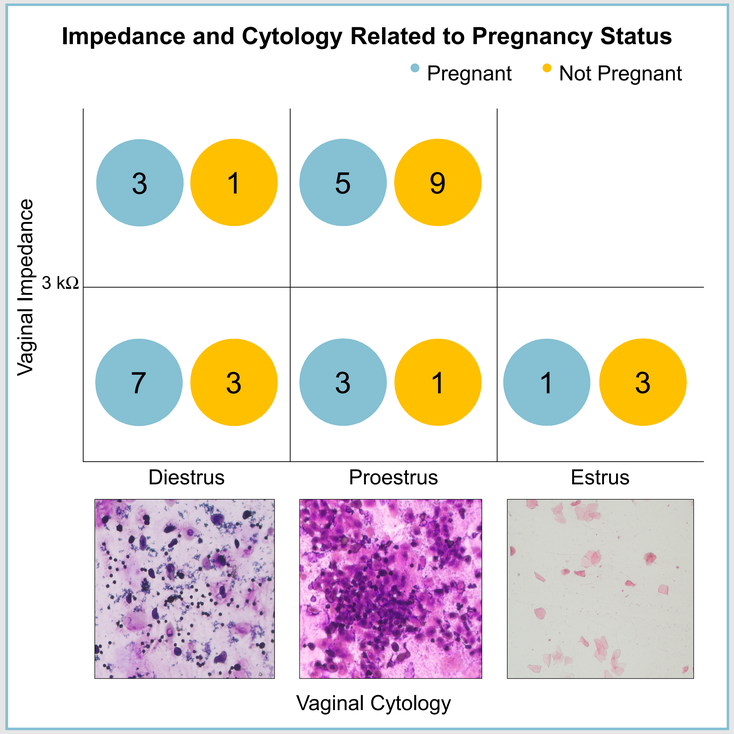

Figure 3. Relationship between vaginal impedance measurement, vaginal cytology reading, and pregnancy. Blue circles denote pregnant females and yellow circles signify not pregnant females at day 10 to 13 post-mating assessment. Numbers within each circle represent the number of females in each grouping. Vaginal cytology images are representative of diestrus, proestrus, and estrus findings. Diestrus is characterized by a predominate population of neutrophils with a mixture of epithelial cells, proestrus is mostly nucleated, commonly clumped, epithelial cells, and estrus appears as all keratinized epithelial cells.

Conclusions

- Vaginal impedance agrees with vaginal cytology on proestrus 78% of the time
  - Rates of false positives and false negatives related to pregnancy are similar
- Neither method is an accurate predictor of pregnancy success in this study

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