Induction of Pseudopregnancy in the Rat by Vaginal Stimulation at Various Stages of the Estrous Cycle [1]

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ABSTRACT Normally-cycling female rats were electrically stimulated at various stages of the estrous cycle to test the effectiveness of a modified probe for the induction of pseudopregnancy. The ability to form decidua served as the criterion of successful response. The probe was constructed to deliver a cervical-vaginal or vaginal-vaginal stimulation rather than the usual cervical-cervical stimulation. The electrical stimulus used consisted of 50 v at 200 pps for a five-second duration. It was found to be effective throughout vaginal estrus but was much less effective during early and late proestrus or early metestrus. Placement of electrodes on the vaginal wall during stimulation was shown to be as effective as placing them on the cervices. As a result, rats may now be made pseudopregnant without visual placement of electrodes thereby simplifying and hastening the stimulation procedure and rendering it more suitable for routine use. Only 20–25 seconds were required to stimulate each animal.

Induction of pseudopregnancy in the rat by mechanical (Long and Evans, '22) or electrical (Shelesnyak, '31) stimulation has required immobilization of the female while in a conscious state (immobilization of the conscious female usually accomplished by tying her down) and use of a speculum and light for observation while stimulating the cervices. Except in the case of cranial stimulation (e.g. Harris, '36), the probe used for electrical induction of pseudopregnancy has invariably consisted of two terminal electrodes 1–2 mm apart which were visually placed on or into the uterine cervices (Shelesnyak, '31; Haterius, '31; Greep and Hisaw, '38; Jacobson, Salhanick and Zarrow, '50; Swingle, Seay, Perlmutt, Collins, Fedor and Barlow, '51; Finn and Keen, '63; Everett, '63; Stone and Emmens, '64). In fact, it has been stated that in prepuberal rats pseudopregnancy is rarely obtained unless the electrodes are placed on the cervical canal (Swingle et al., '51).

In this paper a type of electrical probe is described which eliminates the need for both the speculum and light. This probe, combined with a simple means of restraint, greatly facilitates the procedure and renders the process of stimulation more suited to routine use. The effectiveness of this procedure for the induction of pseudopregnancy has been studied after stimulation at various stages of the estrous cycle.

MATERIALS AND METHODS

Two hundred and twenty-six mature, virgin, Sprague-Dawley females from Charles River Breeding Laboratories were used in this study. Upon arrival, the animals were housed five per cage and maintained on Purina Lab Chow and water ad libitum. Their light day was 7:00 A.M. to 7:00 P.M., and temperature was controlled between 74°C and 77°C.

Daily vaginal smears were taken from each rat, by lavage, for the duration of the experimental period. Females, cycling normally for two complete estrous cycles, were electrically stimulated at various stages of the third cycle (fig. 2). Laparotomy was carried out under ether anesthesia on the fourth day following stimulation at which time the right uterine horn was traumatized by squeezing with artery forceps (Nicholas, '42) in three equidistant places. Sacrifice by decapitation was performed on the fourth day following traumatization. The presence of decidua on the right uterine horn was the criterion used to verify pseudopregnancy.

For stimulation, the animals were rolled into a towel so that the head was covered and only the rump and tail were exposed. The electrical probe was then inserted into the vaginal canal until resistance was met, whereupon each animal received a stimulus of five-seconds duration consisting of

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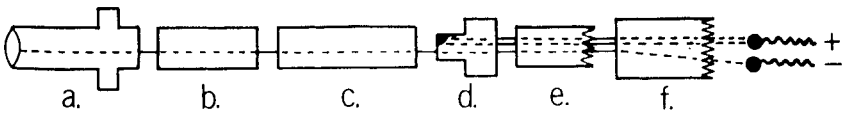

Fig. 1 *Illustration of component used in probe construction:* (a) sterling silver Turret Lug for printed circuit boards (Allied Electronics) — the corners have been rounded, and and a no. 26 Nylclad wire (Allied Electronics) soldered into the tip; (b) a 6 mm piece of polyethylene tubing (PE160); (c) a 1 cm piece of polyethylene tubing to slip over (b); (d) shield grounding ring with 30 AWG, 7 × 38 hookup wire Type E Teflon soldered into it — both (c) and (d) are portions of a 27-7, 50 ohm Microdot assembly (Amphenol); (e) polyethylene tubing (PE 190); (f) polyethylene tubing (PE 240) to serve as handle and to protect union of probe wires to the phonographic pickup wires attached to the induction stimulator.[2] Distance between electrodes when assembled is 1 cm. Complete length of probe is 11 cm. For vaginal-vaginal stimulation, a piece of polyethylene tubing (PE 200) 7 mm long was slipped over the 4 mm tip of the Turret Lug (a). The 3 mm overhang was filled with melted paraffin.

In the author's opinion, any such arrangement will make an effective probe providing water-tight construction is used, the diameter is 5 mm or less, and distance between electrodes is no more than 1.2 cm. The electrode rings must be slightly elevated above the probe body to ensure good tissue contact.

50 v from an induction stimulator [2] delivering approximately 200 pps. After a pause of five seconds, the stimulus was repeated once more.

The vaginal probe used consisted of a blunted terminal electrode followed by a ring electrode at a distance of 10 mm from the first (fig. 1). The current delivered in this case was a cervical-vaginal stimulus rather than the cervical-cervical stimulus used (Shelesnyak, '31).

The effectiveness of a vaginal-vaginal stimulus was tested with the same probe by placing a polyethylene tube, filled with paraffin, over the tip electrode (fig. 1) such that the cephalic ring electrode was 7 mm posterior to the anterior tip of the probe. The voltage and method of stimulation used was otherwise identical to that used for cervical-vaginal stimulation.

Groups were compared by Chi Square (Snedecor, '50).

RESULTS AND DISCUSSION

The results obtained with cervical-vaginal stimulation are summarized in figure 2. The new type probe was effective when used on cycling female Sprague-Dawley rats between vaginal estrus after the disappearance of the epithelial cells of proestrus and the first appearance of leucocytes in metestrus. The incidence of successful pseudopregnancy induction was significantly less during early metestrus "M$^-$" ($P < 0.001$) or prior to estrus "E" ($P < 0.01$). Similarly, pseudopregnancy occurred less frequently if stimulation was given during early proestrus "P$^-$" rather than during early estrus "E$^-$" ($P < 0.05$). Pseudopregnancy was not induced following insertion of the probe without delivery of the electrical current, even though the females were at the most favorable stage of the cycle for pseudopregnancy induction.

Pseudopregnancy can now be readily induced routinely in rats by use of a probe similar to that described. Stimulation is effective if administered at any time between full vaginal cornification and the first appearance of leukocytes — a period consisting of approximately one-quarter of the estrous cycle (see review by Mandl, '51). Hence, females too late in their estrous cycle to be bred to vasectomized males will still respond to this means of stimulation. The method is simple since no speculum or light is required, and since only a rolled towel is necessary for restraint. The total time required is 20–25 seconds per animal.

The statement that both electrodes need to be placed on the cervices (Swingle et al., '51) does not apply to the adult female, since when both electrodes touched only the vaginal wall, all the ten females so stimulated formed decidua.

The probe used to date consisting of two parallel metallic electrodes 1–2 mm apart gave a maximal response during physiological estrus (Shelesnyak, '31; Haterius, '33) when the vaginal smear contained

[2] Induction Stimulator Model 1049, Harvard Apparatus Co., Dover, Massachusetts.

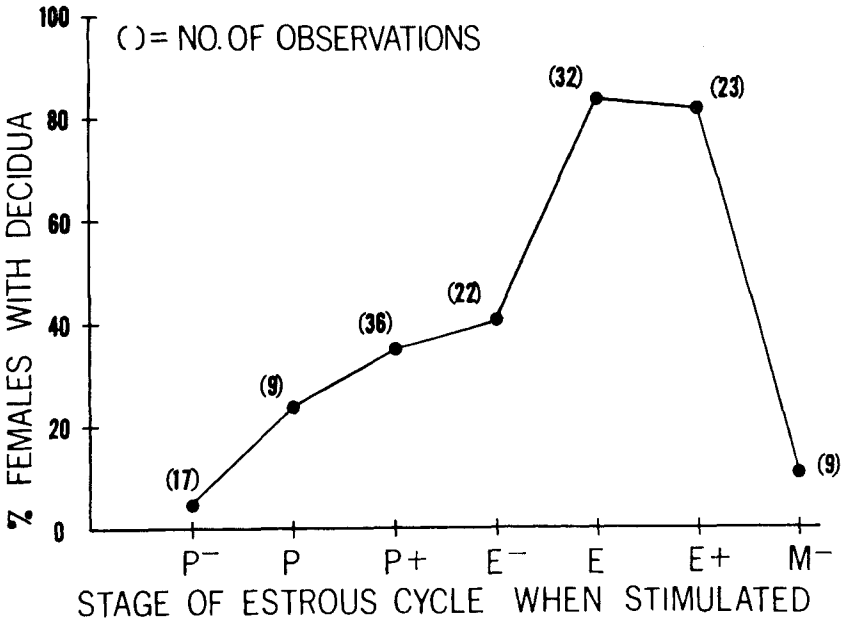

Fig. 2 *Pseudopregnancy induction by cervical-vaginal stimulation at various stages of the estrous cycle.*

P^- — vaginal smear consists of epithelial cells, keratinized and non-keratinized cells as well as a few leukocytes (see Staples and Geils, '65) — approximately 4–6 hours prior to the complete epithelial smear of proestrus.

P — vaginal smear consists mainly of small round epithelial cells — the typical proestrous smear.

P^+ — still more epithelial cells than pre-keratinized and keratinized cells in vaginal smear.

E^- — epithelial cells still present, but vaginal smear consists largely of pre-keratinized, basophilic cells.

E — virtually no epithelial cells remaining, and the smear consists of a mass of keratinized cells (acidophilic) — some pre-keratinized cells present particularly early in this stage.

E^+ — the smear consists mainly of acidophilic, shriveled keratinized cells, some pre-keratinized cells; and towards the end of this stage, epithelial cells again return.

M^- — leucocytes again present in the smear with the epithelial and the keratinized cells.

The distance allotted to each group is not proportional to the duration of each stage of the estrous cycle.

both epithelial and cornified cells. On the other hand, the probe used here gave maximal response after ovulation (Young, Boling and Blandau, '41) at a time when physiological estrus had ceased. The reason for this difference is not known. This finding is not, however, unprecedented in that others have also found the rat susceptible to pseudopregnancy induction after physiological heat. Meyer, Leonard and Hisaw ('29) induced pseudopregnancy in 69% of rats stimulated with a glass rod during Long and Evans Stage 2 or 3 of the estrous cycle, which is during early and late vaginal cornification. Similarily, Sydnor ('45) was able to induce pseudopregnancy with LTH more effectively at the time of ovulation than at proestrus.

The stimulation described was fairly close to the minimal threshold necessary for pseudopregnancy induction by this technique; for it was noted that if the voltage output of the battery was reduced by 15%, the incidence of pseudopregnancy induction during vaginal estrus was reduced by approximately 85% (data not included in fig. 2). Even this reduced voltage caused the tail to become erect and the hind quarters to convulse upon stimulation. Hence, these criteria alone were not

sufficient for postulating a successful stimulation for pseudopregnancy induction. Woolley and Timiras ('62) also found that the electrical induction of convulsions during estrus was not synchronous with pseudopregnancy induction, for pseudopregnancy did not occur after applying sinusoidal current at 60 pps to electrodes placed over the eyes of mature female rats.

Fertilized ova transferred to rats made pseudopregnant by use of this probe do implant and normal pregnancy ensues (Staples, '65).

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