Effect of bilateral oophorectomy on wound healing of the rabbit vagina

We aimed to assess the effect of bilateral oophorectomy on vaginal wound healing in three groups of New Zealand White rabbits (24 rabbits each). Group 1 underwent bilateral oophorectomy, group 2 underwent a sham surgery, and group 3 served as control. Standardized vaginal tissue specimens were harvested and assessed for wound and scar surface area and tensiometric analysis before wounding, and sequentially thereafter, showing that vaginal wound closure, scar contraction, and recovery of biomechanical properties are significantly slower in oophorectomized rabbits. (Fertil Steril® 2011;95:1467–70. ©2011 by American Society for Reproductive Medicine.)

Key Words: Biomechanical properties, estrogen deficiency, menopause, oophorectomy, tensile energy, tensile strength, vagina, wound healing, Young's modulus

The volume of pelvic reconstructive surgeries increases each year due to the widespread prevalence of pelvic organ prolapse and incontinence within the aging female population. Up to 11% of American women will undergo major prolapse or incontinence surgery by age 80 years, and 29% of these women will require repeat surgery due to recurrence of their symptoms (1). The physiological surgical wound healing process in the vagina is a major determinant of patients' recovery from reconstructive pelvic surgeries and in the success or failure of these procedures. Because a large proportion of these surgeries are being performed in postmenopausal women, it is essential to understand how estrogen (E) deficiency affects this process. There is striking evidence from animal studies that Es play a crucial role in cutaneous wound healing and that repair is significantly delayed in its absence (2–5). Reduced E levels have major downstream effects on cellular and tissue responses to injury, including impaired cytokine signal transduction, unchecked inflammation, and altered protein balance (2–5). At present only scant data have been published regarding the role of Es in the wound healing process in the vagina of either animals or humans. In the current study we aimed to assess the effect of E deficiency on biomechanical properties of the vaginal surgical wound healing process using a rabbit model.

Seventy-two sexually mature, pure bred, nulliparous, female, New Zealand White rabbits aged 35–40 weeks, weighing 4.0–4.5 kg, were used for this study. In general, rabbits do not ovulate spontaneously, and therefore maintain a fairly stable hormonal status (6). New Zealand White rabbits have been shown to have a capacious vagina, which is amenable to vaginal surgery (7). Rabbits were housed in individual cages in a central animal care facility, maintained on a 12-hour light–dark cycle, and given free access to standard rabbit chow and water. The animal care facilities were maintained in accordance with federal guidelines. The study was approved by the Institutional Animal Care Utilization Committee. Of 72 rabbits, 24 were randomly assigned to serve as experimental animal models of the equivalent of human surgical menopause (group 1) (8). These animals underwent bilateral oophorectomy at Covance Research Products Laboratories (Denver, PA) using IM ketamine-HCl (50 mg/kg) and xylazine (5 mg/kg) anesthesia. They also received 100 mg of cefazolin immediately preoperatively for antibacterial prophylaxis. The abdominal skin was prepped with povidon-iodine solution. After infiltrating the lower abdomen with lidocaine, a 5-cm lower midline abdominal incision was created. The ovarian vessels were ligated with 3-0 silk ties and the ovaries were removed. After ensuring adequate hemostasis, the abdominal cavity was closed in layers with 2-0 polyglycolic acid sutures. All animals received routine pain control with buprenorphin hydrochloride 0.15 mg two times per day for 2 days after wounding. Another 24 rabbits underwent a sham operation consisting of a horizontal 5-cm lower midline abdominal skin incision using the same anesthetic, analgesic, and antimicrobial measures as mentioned previously (group 2). The remaining 24 rabbits served as controls (group 3). Eight weeks later, vaginal

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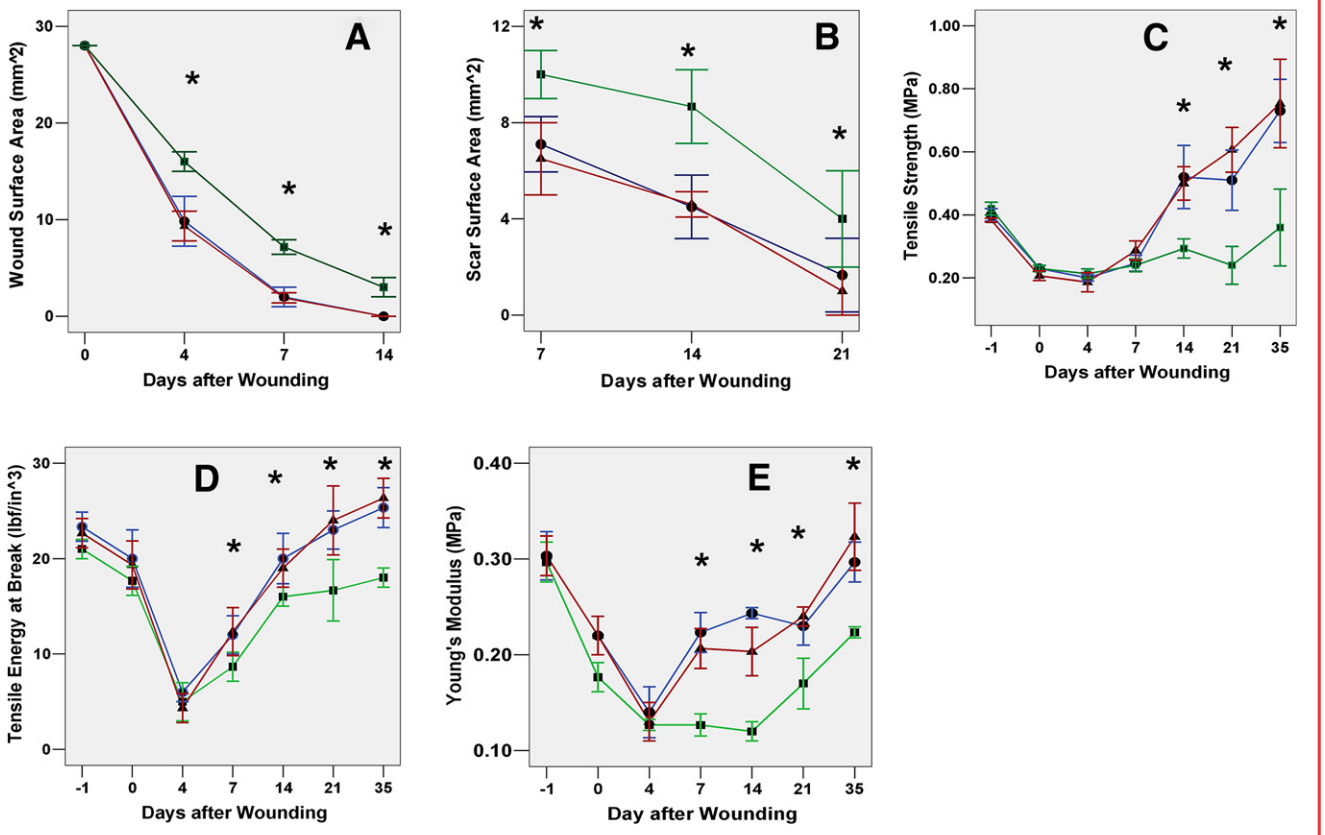
FIGURE 1

Changes in the wound's surface area (**A**), scar's surface area (**B**), wound's tensile strength (**C**), tensile energy to break (**D**), and Young's modulus (**E**) with time in normal rabbits (*circles*), after sham surgery (*triangles*), and after bilateral oophorectomy (*squares*). Day -1 = before wounding; day 0 = immediately after wounding. Values are presented as means ± SD. *$P < .05$ for comparisons between oophorectomized and normal rabbits.



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wounding was performed in all rabbits, in a random order, by a single surgeon (Y.A.). Animals were anesthetized and prepped. Standardized 6-mm full-thickness circular segments were excised from the external vagina using a dermatologic biopsy punch. Wounding was performed on one of the lateral vaginal walls, 1.5 cm proximal to the vaginal introitus under direct visualization. Hemostasis was achieved using local pressure, as needed. A triple antibiotic ointment (polymyxin B, neomycin, and bacitracin) was applied to all wounds immediately after wounding. All animals received routine postoperative pain control as described previously. Animals were euthanized by lethal IV injection of pentobarbital (100 mg/kg) before wounding (day -1), and at 0, 4, 7, 14, 21, and 35 days after wounding. At each time point, 9 (3 from each group) or 12 (4 from each group) rabbits were euthanized. The rabbits' vaginal wounds and scars were inspected by an examiner blinded to the rabbit's treatment group. The wound's diameters were recorded at two separate axes perpendicular to one another and their average value calculated. The wound's surface area was then calculated using the equation $s = \pi r^2$, where s = wound's surface area and r = the average wound's radius, calculated as half the average diameter. Wound closure was defined as the reduction in the wound's surface area. Scar tissue was identified macroscopically, and its surface area calculated in a similar manner as described previously. At each time point, immediately after the animals were euthanized, 2.6-cm by 1.6-cm rectangular vaginal tissue strips with the experimental wound in their center were excised en-bloc at a full-thickness depth using a custom-made instrument containing stainless steel skin graft knife blades. The wound was centered and its thickness measured using a standardized soft tissue micrometer. Tensile properties of these strips were measured by an examiner blinded to the rabbit's treatment group using an Instron 5544 tensiometer (Instron, Canton, MA) with a 500 N load cell, using previously reported methods (9). The strips were gripped next to the wound edges, parallel to the longer axis of the rectangular specimen, between sandpaper in sinusoidal clamps. A tensile preload of 0.1 N was applied. The tissue specimen was then preconditioned 10 times with a sine wave at a rate of 0.6 N/s with a maximum load of 0.5 N. After the tissue was examined for irregularities due to the preload or preconditioning, the specimen was loaded in tension to a failure rate of 0.25 mm/s, which is approximately 100% per minute strain rate based on the cut-out region, to generate stress versus strain curves. Tensile strength, tensile energy to break,