Intravaginal impedance and sexual behavior of ovariectomized goats given estrogen alone or in combination with progesterone

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ABSTRACT: Intravaginal impedance (IVI) fluctuates during the goat estrous cycle. To understand which ovarian steroids are responsible for IVI changes and whether IVI variations are associated with precopulatory and copulatory behaviors, 8 ovariectomized females were assigned to 4 treatments in a 4 × 4 Latin square replicated over four 8-d periods. The treatments were as follows: progesterone plus estradiol-17β (P4 + E2), oil plus estradiol-17β (E2), progesterone plus oil (P4), or oil (OIL). Daily IVI measurements at the vaginocervical junction were taken at 1 and 70 KHz. Progesterone was given on d 2 and 3. Estradiol was given in the evening of d 5. On d 1 to 8, goats were group-exposed to a sexually experienced male and observed for the expression of sexual behaviors. On d 6 and 7, IVI was less when goats received P4 + E2 or E2 compared with goats given P4 or OIL (P < 0.05). Impedance measured at 1 kHz tended to remain lower on d 8 in P4 + E2-treated females compared with those given P4 or OIL (P < 0.055). Like previous results, P4 + E2 or E2 treatment induced behavioral estrus; 5 of 8 P4 + E2-treated and 5 of 8 E2-treated females were sexually receptive on d 6. On d 7, although IVI remained low and 2 of 8 P4 + E2-treated goats and 4 of 8 E2-treated goats remained sexually receptive, no additional females were in estrus. No IVI decreases and no estrous behavior were observed in goats given P4 or OIL. This experiment demonstrated that E2 initiates the periestrous drop in IVI, and P4 may delay baseline return.

Key words: estrogen, estrus, goat, impedance, progesterone, vagina

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INTRODUCTION

During the estrous cycle, ovarian steroids exert histological and biochemical effects on the reproductive tract. For example, fluctuations in ovarian steroids alter vaginal mucus composition (Wolf et al., 1978; Adams, 1981), increase edema within the reproductive tract (Rhen et al., 2003), and modulate uterine secretions (Spencer and Bazer, 2004). These changes alter electrical properties of the cell membranes and intra- and extracellular spaces (Schwan, 1957; Adam et al., 1981). Thus, ovarian endocrine activity can be indirectly monitored by measuring impedance, which is the ability of the reproductive tract to resist the flow of an externally applied, weak electrical current (Lehrer et al., 1991, 1992; Bartlewski et al., 1999).

Intravaginal impedance (IVI) fluctuates during the estrous cycle of domestic animals (Edwards et al., 1992). In cattle, the lowest IVI readings were 8 to 20 h after the onset of standing estrus (Aizinbudas and Doviltis, 1963; Schams and Butz, 1972; Ezov et al., 1990). The lowest IVI reading in most ewes coincided with standing estrus and occurred just before standing estrus in the remainder (Feldman et al., 1976; Lehrer et al. 1979). Similarly, in goats, IVI drops 2 d before standing estrus and returns to baseline by 2 d postestrus (Rezac et al., 2001). It is unclear whether cyclic IVI changes are due to increased estradiol (E2) production during the periovulatory period (Schams et al., 1977), changes in progesterone (P4), or the E2:P4 ratio (Bartlewski et al., 1999). Moreover, it is unknown whether the presence of both steroids is necessary for the IVI changes observed in gonad-intact animals. To our knowledge, no one has directly tested the effects of E2, P4, or the combination on IVI changes.

Here, ovariectomized goats were used to assess the contributions of P4, E2, or both to IVI changes and to determine the temporal relationship between those

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changes and the occurrence of precopulatory and copulatory behaviors.

MATERIALS AND METHODS

General Methods and Animals

All animal maintenance and research procedures were in accordance with regulations established by Rutgers University Animal Care and Facilities Committee.

This experiment was conducted from January 6 to April 11, 2005, at the Cook College Small Animal Research Farm in New Brunswick, New Jersey (40°28′34″N, 74°26′19″W). Ambient day length ranged from 10.4 to 14.1 h. Eight adult French-Alpine previously ovariectomized (i.e., for 1 yr before the beginning of this experiment) females and 2 adult gonad-intact French-Alpine males were used in this experiment. All females were 1 to 2 yr of age before ovariectomy and were 3 to 4 yr of age at the beginning of the study. Body weights of females ranged from 40 to 60 kg. All animals had extensive prior sexual experience both pre- (unpublished results) and postovariectomy (Imwalle and Katz, 2004a,b). The females were housed together in a closed barn with natural lighting and had free access to an outdoor field. The males were group-housed with other males in a separate open-sided barn, also with outdoor access and natural lighting. All goats were fed a complete pelleted ration that met the NRC (1981) standards for goats and grass hay along with ad libitum access to water and a mineral block.

Treatments and Experimental Design

Estradiol-17β, and P₄ were injected s.c. (Steraloids Inc., Newport, RI). Estradiol-17β (50 μg/mL) was dissolved in ethanol and then in sesame oil at a ratio of 1 part ethanol to 20 parts sesame oil (vol/vol). Progesterone (10 mg/mL) was dissolved in benzyl benzoate and then placed in sesame oil 1:10 (vol/vol). Prior work in our laboratory (Kaplan and Katz, 1994; Billings and Katz, 1997, 1998) has shown that both the dose and timing of administration of P₄ and E₂ as described in this experiment elicits sexual behavior similar to that observed in gonad-intact, sexually experienced female goats. Briefly, this model involves the administration of 10 mg of P₄ 72 h before E₂, 5 mg of P₄ 48 h before E₂, and 50 μg of E₂ 14 h before the reproductive behavior test.

A Latin square arrangement of 4 treatments × 4 repetitions, each repetition lasting 8 d, was used in this experiment. Seven days separated each repetition to allow for clearance of the hormone treatments (Billings and Katz, 1997). During each repetition, 2 goats represented each treatment. Each goat received each treatment once. Thus, during each repetition, female goats were randomly assigned to receive 1 of 4 treatments: P₄ + E₂, P₄ on the evenings of d 2 (10 mg) and d 3 (5 mg) and 50 μg of E₂ on the evening of d 5; E₂, sesame oil on the evenings of d 2 (1.0 mL) and d 3 (0.5 mL) and 50 μg of E₂ on the evening of d 5; P₄, P₄ on the evenings of d 2 (10 mg) and d 3 (5 mg) and sesame oil on the evening of d 5 (1.0 mL); OIL, sesame oil on the evenings of d 2 (1.0 mL) and d 3 (0.5 mL) and sesame oil on the evening of d 5 (1.0 mL).

IVI Measurements

Impedance measurements were taken on the mornings of d 1 to 8. A custom-made 20-cm-long probe, consisting of 2 medical-grade, stainless steel ring electrodes at one end (3-mm high × 12-mm diam., 1 cm apart), was inserted into the vagina. Intravaginal impedance at the vagino-cervical junction was measured using a custom-made impedometer (Shlomo Wollstein, Rehovot, Israel) at 1 KHz (indicating tissue extracellular space) and 70 KHz (indicating extra and intracellular spaces) using 1.5-mA current (Gambini et al., 1980; Lewis et al., 1989).

Sexual Behavior Test

Females were group-tested for sexual behavior each morning of d 1 to 8. One male goat was placed into the pen of the females for 5 min. Females exhibiting standing estrus were removed from the pen. Then, the first male was removed, and the second male was placed in the pen for 5 min. If the second male detected any additional females in standing estrus, those females were also removed. This process was repeated so that both males were given two 5-min intervals to detect females in standing estrus. Between each 5-min test, the resting male was allowed to view the sexual interactions of the other male (Price et al., 1984). The order of male introduction was alternated across days of the experiment. Moreover, all males in this experiment had a high serving capacity (range of 5 to 7 ejaculations in 15 min; Imwalle and Katz, 2004b). The same 2 observers scored each test and were blind to the treatment.

A sexual behavior score based on an ordinal scale was calculated for each female. Similar methodologies have been used in other animal models to describe differences in the relative intensity of a behavior (Lightbody and Weatherhead, 1987; Ward, 1988; Lehner, 1992). A female was assigned 1 point if the male sniffed her anogenital region. Two points were assigned for each of the following that occurred: a female wagged her tail (i.e., showed proceptive behavior; Imwalle and Katz, 2004a), if the male courted her, or if the male attempted to mount her. Females were given 3 points if they exhibited standing estrus or if the male had a successful ejaculation, indicated by a period of rapid thrusting, followed by a single deep thrust and immediate dismount, with a subsequent lack of interest in the female (Maina and Katz, 1999). Points were awarded for a single occurrence of a particular event during the test period. For example, if a female was
sniffed (1 point), wagged her tail (2 points), showed standing estrus (3 points), and the male ejaculated (3 points), she received a score of 9 points. Accrued points for each female were then summed to obtain a sexual behavior score for each day. Although sniffing of females is not a sex-specific behavior and may be related to social behaviors, we included chemoinvestigation in our analysis, because these behaviors are seen more frequently in bulls (Estes, 1972; Hradecky et al., 1983) and male goats (Delgadillo et al., 2002) if the female is in estrus.

**Statistical Analysis**

All data analyses were conducted using NCSS software (NCSS Statistical Software, Kaysville, UT). The IVI data were analyzed by repeated-measures ANOVA. Main effects in the model were animal, treatment, day, and treatment × day. To test for significant treatment effects (among-subjects analysis), animal within treatment was the error term. To test for significant day and day × treatment interaction (within-subject analysis), the mean square error was used. If a significant \( (P < 0.05) \) treatment × day interaction was detected, post hoc differences in IVI for each day were then compared using the Tukey-Kramer multiple comparison test \( (P < 0.05) \). Unless a significant treatment × day interaction was detected, there was no justification for making additional pairwise comparisons at each day.

Because the behavioral data are by definition nonparametric and were therefore scored on an ordinal scale, we used Friedman’s 2-way ANOVA to detect differences between treatment and day (Lehner, 1996). The Friedman 2-way ANOVA uses \( \chi^2 \) analysis to test the null hypothesis that there is no difference among the sexual behavior scores of goats, given their respective treatments. If significant differences were detected, Dunnett’s multiple comparison test was used to determine which pairs of samples differed significantly (Lehner, 1996).

**RESULTS**

**E2 Initiates and P4 May Sustain the Drop in IVI Associated With Estrus**

Eight of 8 females given E2 and 7 of 8 females given P4 + E2 experienced at least a 30% decline in IVI on d 6 (i.e., the expected day of standing estrus). In contrast, no OIL-treated and no P4-treated females showed similar declines in IVI levels. Analysis of variance revealed a treatment × day interaction for the 1 KHz frequency \( (P < 0.001) \). Impedance measurements did not differ among treatments on d 1 to 5. On d 6 to 8, IVI was lower in goats given E2 or P4 + E2 compared with OIL- or P4-treated goats \( (P < 0.05; \text{Figure 1, panel A}) \).

Analysis of variance revealed a treatment × day interaction for the 70 KHz frequency \( (P < 0.001) \). Impedance measurements did not differ among treatments on d 1 to 5. On the morning of d 6 and 7, IVI was lower in goats given E2 or P4 + E2 compared with OIL- or P4-treated goats \( (P < 0.05; \text{Figure 1, panel B}) \). However, on d 8, there was a tendency for IVI only at the 70 KHz frequency to be less in P4 + E2-treated females with
respect to all other treatments ($P = 0.055$; Figure 1, panel B).

**Both E$_2$ and P$_4$ + E$_2$ Treatments Increased Sexual Behavior Score Compared With Females Given P$_4$ or OIL**

No goat given P$_4$ or OIL showed a drop in IVI or standing estrus at any time of the experiment. In contrast, 5 of 8 females given P$_4$ + E$_2$ and 5 of 8 females given E$_2$ showed standing estrus, and 9 of these 10 females that displayed standing estrus also showed a drop in IVI. On d 6, five of 8 females in both E$_2$ and P$_4$ + E$_2$ treatments displayed standing estrus. On d 7, four of 8 E$_2$-treated and 2 of 8 P$_4$ + E$_2$-treated females displayed standing estrus. On d 8, although there were no differences among groups, females in the P$_4$ + E$_2$ and E$_2$ groups did not display standing estrus; however, they also did not overtly avoid the males.

The $\chi^2$ value of the Friedman 2-way ANOVA indicated that there is a difference ($\chi^2_{1,13} = 1,340.8; P < 0.001$) in behavior score in goats among the different treatments. Furthermore, on d 1 to 5, although males repeatedly courted and sniffed females, there were no differences in sexual behavior score among groups, and females in all groups showed no interest in the males. However, on d 6 and 7, females given P$_4$ + E$_2$ or E$_2$ received greater sexual scores compared with females given P$_4$ or OIL treatments ($P < 0.05$; Figure 1, panel C).

**DISCUSSION**

Using the ovariectomized goat, we present new information on sex steroid-driven changes in IVI. Both P$_4$ + E$_2$ and E$_2$ treatments stimulated a drop in IVI in all females, whereas no IVI decrease was measured in P$_4$- or OIL-treated animals. At the 1 KHz frequency, IVI dropped on the morning of d 6 and remained low on d 8. At the 70 KHz frequency, IVI tended to remain lower in P$_4$ + E$_2$-treated females on d 8 compared with other groups. To our knowledge, this is the first report to quantitatively associate a sexual behavior score with changes in IVI. Taken together, the results of this experiment suggest that E$_2$ alone triggers the drop in IVI, and P$_4$ may be responsible for sustaining this drop during the periestrus period.

Both P$_4$ + E$_2$ and E$_2$ treatments caused a sharp drop in IVI on d 6 at both frequencies, suggesting that E$_2$ treatment is primarily responsible for the initial alteration in reproductive tract characteristics during the periestrus period. These results support work done in gonad-intact cattle that suggested that increased E$_2$ production during the periovulatory period was responsible for the drop in IVI (Schams et al., 1977). On the other hand, the current results do not support the claim that P$_4$ alone, or in conjunction with the E$_2$:P$_4$ ratio, is responsible for changes in IVI in gonad-intact sheep (Bartlewski et al., 1999), because the P$_4$ treatment in the current study had no effect on IVI measurements.

In other species, E$_2$ has been implicated as the primary facilitator of physiological changes to the vaginal epithelium. For example, in cynomolgus monkeys (Robinson et al., 1996) or rats (Suguita et al., 2000), either P$_4$ + E$_2$ or E$_2$ treatments rapidly increased amounts of vaginal epithelium compared with control animals. This current work agrees with literature in primates and rodents by demonstrating that E$_2$ or P$_4$ + E$_2$ indirectly alter the reproductive tract of females (as measured by the sustained drop in IVI). Furthermore, because there was a tendency for the IVI values of the P$_4$ + E$_2$ treatment to suppress IVI on d 8, we hypothesize that P$_4$ from the prior cycle may enhance estrogen-induced changes in the reproductive tract and may influence gamete transport and fertility. Further work is needed to assess this effect (e.g., P$_4$ treatment for a longer period).

The drop in IVI at the 1 KHz frequency was sustained over a longer period in goats compared with the drop at the 70 KHz frequency. This finding suggests that the resumption of preestrus extracellular space volume, which includes the vaginal mucus, is slower than the resumption of intracellular space. The drop in IVI and return to baseline lasted approximately 3 d. This is shorter than the 4-d interval reported in previous work using gonad-intact goats (Rezac et al., 2001). We hypothesize that the temporal difference between our work and the work of Rezac et al. (2001) may be due to differences in the experimental model. For example, circulating P$_4$ and E$_2$ concentrations rise and fall rapidly when supplied exogenously (Billings and Katz, 1997) compared with the sustained releases observed in a gonad-intact, cycling goat (Zarkawi and Soukouti, 2001). Regardless, the delay in baseline return in gonad-intact goats (Rezac et al., 2001) agrees with our finding that ovariectomized goats given P$_4$ + E$_2$ showed a delay in return to baseline at the 70 KHz frequency (Figure 1, panel B).

The most conservative criterion of female ruminant sexual behavior has historically been the expression of standing estrus, the period during the estrous cycle in which the female remains motionless when mounted by a conspecific. Although the expression of standing estrus may be the benchmark measurement, efficiency of estrous detection is often poor (Senger, 1994), and standing estrus varies in intensity (Dransfield et al., 1998). Moreover, intensive agriculture practices (e.g., concrete flooring or free stalls) may interfere with the desire of the animals to display estrous behavior (Kerbrat and Disenhaus, 2004). Thus, several laboratories (Van Vliet and Van Eerdenburg, 1996; Kerbrat and Disenhaus, 2004) have advocated a more inclusive approach to estrous detection. This approach uses the recording of the expression of secondary sexual behaviors such as sniffing, chin resting, attempted mounting, and escapes from mounting as well as the expression of standing estrus. Our point system as used in this work supports this inclusive approach.
In the current work, both $P_4 + E_2$ and $E_2$ treatments increased sexual behavior scores on d 6 and 7 compared with females given $P_4$ or OIL. The increased scores are reflective of increases in the overall stimulus value of the female (i.e., attractivity, proceptivity, and receptivity) during the onset of behavioral estrus (Beach, 1976). We noted an increase in these scores during the period when IVI began to drop, hinting that changes in the reproductive tract (as observed by the drop in IVI) may also contribute to increases in sexual behavior indices (Beach, 1976). It is interesting to note that although most goats given $E_2$ or $P_4 + E_2$ showed a drop in IVI, fewer goats showed standing estrus. To better associate changes in IVI to changes in sexual behavior, it is possible that more frequent IVI measurements, in conjunction with sexual behavior scoring, are necessary. On the other hand, the fact that goats showing a decrease in IVI were not detected in estrus suggests that once-daily IVI measurements, as conducted in this study, might substitute the need for more frequent observations for standing estrus for AI purposes. In sum, our results raise new comparative questions on the endocrine control of expression of sexual behavior and changes in IVI in goats.

**LITERATURE CITED**


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