Impact of uremia on female reproductive cyclicity, ovulation, and luteinizing hormone in the rat

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Background. Impaired reproductive function accompanies chronic renal insufficiency (uremia) in both the human and experimental animal. Clinical hypogonadism occurs in both genders. The present studies were designed to investigate possible anti-ovulatory effects of uremia in the female rat, a species that produces multiple ova during the normal estrous cycle. Possible disruption of hypothalamic-pituitary-ovarian regulation is further highlighted by attenuation in the preovulatory LH surge. These results provide a basis for further studies of neuroendocrine pathophysiology in a rodent model of uremia-associated ovulatory disruption.

Methods. Renal insufficiency (uremia) was induced by 5/6 nephrectomy. Two control groups comprised sham-operated animals fed ad libitum (sham) or pair-wise with the uremic animals (pair-fed). Estrous cycles were determined by cytology of vaginal lavage. We examined concomitant changes in the preovulatory luteinizing hormone (LH) surge by radioimmunoassay (RIA), immunoradiometric assay (IRMA), and bioassay. Repetitive LH measurements were made from blood samples taken by intra-atrial catheter throughout the afternoon and evening of proestrus. The following morning (estrus), ovaries were collected, and ova were enumerated per oviduct.

Results. Experimentally uremic animals manifested a three-fold elevation of plasma creatinine and urea nitrogen and concomitantly a more than 50% impoverishment of ova production. Analyses of a large group of animals (N = 83) by RIA revealed uremia-associated attenuation of the preovulatory LH surge. Further measurements of the preovulatory LH surge by independent IRMA and LH bioassay (N = 26) corroborated this attenuation. Additional experiments indicated that these hormonal changes, but not changes in ovulation, might further reflect modulation of LH release by the anesthesia used in the preparative nephrectomy and catheterization surgeries. When normalized to body weight, the ovaries of uremic rats were found to weigh more than those of either the sham or pair-fed animals.

Conclusions. The present experiments take advantage of an experimental uremic model to document a consistent decrease in the number of ova released during estrus in the uremic animal. Possible disruption of hypothalamic-pituitary-ovarian regulation is further highlighted by attenuation in the preovulatory LH surge. These results provide a basis for further studies of neuroendocrine pathophysiology in a rodent model of uremia-associated ovulatory disruption.

A clear association exists between the adequacy of kidney function and the integrity of the reproductive system in both sexes. In male animals [1] and in human males [2, 3], renal insufficiency can elicit marked hypogonadism. In the human male, diminished libido, variable infertility, impotence, and clinical feminization (for example, gynecomastia) are well recognized [4-7]. Testicular weights are significantly lower in uremic animals than in ad libitum-fed controls or pair-fed (food-restricted) animals [8]. Significant abnormalities in testicular histology, reduced testosterone secretion, and impaired growth and maintenance of androgen-dependent secondary sex organs also occur in uremic rats [8]. Normal Leydig-cell response to human chorionic gonadotropin (hCG) in vivo and in vitro in some studies would point to possible hypothalamic-pituitary dysfunction [1].

Uremia can also be accompanied by reduced basal luteinizing hormone (LH) concentrations in otherwise intact male rats despite a decline in circulating testosterone concentrations, which might be expected to stimulate LH secretion because of withdrawal of androgenic negative feedback. After gonadectomy, uremia resulted in an excessive increase in serum LH concentrations, which was not due to retention of immunoreactive fragments or undernutrition [9, 10]. An interpretation of hypothalamic dysfunction under these conditions was further supported by a lack of LH responsiveness to naloxone infusion and an alteration in sensitivity to steroid-negative feedback.

A recent study monitored pituitary secretion of LH in parallel with hypothalamic secretion of gonadotropin-releasing hormone (GnRH) by way of pituitary microdialysis in adult male rats [11]. Despite higher mean circu-
Lating LH concentrations in gonadectomized uremic rats, the LH secretory burst frequency and amplitude were actually depressed in uremia. LH hypopulsatility was attributed to a 38% decrease in the total GnRH secretion rate estimated by microdialysis in vivo. These data correspond well with an independent report of a 40% reduction in GnRH production by in vitro superfused hypothalamic explants from uremic male rats [12].

Clinical studies in women have also indicated the possibility of hypothalamic anovulation in uremia [13]. Severely uremic women typically have oligomenorrhea or amenorrhea. Although serum LH concentrations are slightly elevated and rise after clomiphene (anti-estrogen) administration, ovulation is not induced by treatment with ethinyl estradiol. Such observations suggest preservation of secretion and estrogenic negative feedback but disruption of positive estrogen feedback. One pathophysiological consideration is the associated hyperprolactinemia evident in uremic women [14]. The apparent lack of prolactin secretory responsiveness to several inhibitory or stimulatory agents administered under these conditions could indicate primary pituitary dysfunction, without or with a concomitant hypothalamic defect. In contrast to these clinical insights in uremic women, few data exist in experimental renal failure in female animals.

With these considerations in mind, the present study was designed to investigate the fundamental effect of renal insufficiency on estrous cyclicity, the preovulatory surge of LH, and concomitant ova released in the female rat with experimentally imposed uremia.

**METHODS**

All animal procedures were conducted after approval of the protocol by the Institutional Animal Care and Use Committee (IACUC) of Virginia Commonwealth University (VCU).

Adult female Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA, USA) were received at approximately 70 days of age (150 to 175 g body weight) after they had been screened for four-day estrous cycles. Animal quarters were controlled for temperature (21 to 23°C) and light [14:10 light:dark, lights on at 05.00 hours Eastern Daylight Time (EDT)]. Estrous cycles were monitored by cytological evaluation of vaginal lavage. After a six-day adaptation period, animals were separated into three weight-matched groups. One group underwent 5/6 nephrectomy to produce chronic renal insufficiency (“uremic”), and two control groups were either sham operated and fed ad libitum (“sham”) or sham operated and pair-fed (“pair-fed”) with the uremic animals. Nephrectomy was accomplished via a two-stage operation. In the first stage, the left kidney was exteriorized through a flank incision and decapsulated, and the upper and lower thirds were removed. One week later, the second stage involved complete removal of the right kidney. Sham surgery involved exteriorization and decapsulation of each kidney at the appropriate time. Penicillin G procaine suspension (Wycillin; Wyeth Labs, Philadelphia, PA, USA) was applied to the wound, and the animals were allowed to recover on a heating pad until fully ambulatory. Each pair-fed animal was matched to a 5/6 nephrectomized animal and received only as much food as the uremic animal consumed. Tap water was available to all animals ad libitum.

Five replicates of the experiment were performed. In the first replicate, only estrous cyclicity and the preovulatory LH surge were studied. Repeated blood samples were obtained via an intra-atrial catheter [15], which had been implanted on the day prior to expected proestrus. Animals were then verified to be in proestrus by vaginal smear. Those that failed to show proestrus vaginal cytology followed by an estrous smear on the next day were not included in the study. Blood samples (0.3 mL) were collected into heparinized syringes at hourly intervals from 14.30 to 22.30 hours. Plasma was separated by centrifugation. Red blood cells were resuspended in saline and reinjured after each sample. The first sample was replaced with saline.

In the subsequent four experiments, estrous cyclicity, preovulatory LH release, the number of ova released, and the weights of the ovaries were evaluated. In all of the repetitions except one, the nephrectomies and catheterizations were performed using ether under a fume hood. In one repetition, brevital (Sodium methohexital; Eli Lilly, Indianapolis, IN, USA) was used for the surgeries.

For the first four repetitions, LH was assayed by double antibody radioimmunoassay (RIA) using kits provided by Dr. A.F. Parlow and the National Hormone and Pituitary Program of NIDDK. Reference preparation was rat LH RP-2. Intra-assay and interassay coefficients of variation were 7.5 and 9.8% at 0.5 and 0.625 ng/tube, respectively.

For the last repetition, immunoradiometric assay (IRMA) and bioassay of LH were performed. For the IRMA, LH was measured in 25 μL plasma samples by a modified two-site sandwich immunoassay [16] using double monoclonal antibodies against bovine LH [17] and human LHβ subunit, as described previously [16]. The monoclonal capture antibody (no. 5303; Medix, Kauniainen, Finland) was biotinylated and immobilized on avidin-coated polystyrene beads (7 mm; Nichols Diagnostic Institute, San Juan Capistrano, CA, USA). The tracer antibody (provided by Dr. Janet Roser, Department of Animal Science, University of California, Davis, CA, USA) was iodinated by the chloramine-T method and purified by Sephadex G-50 chromatography. Rat LH RP-3 (National Hormone and Pituitary Program)
Table 1. Values of plasma creatinine and urea nitrogen at least 14 days after 5/6 nephrectomy

<table>
<thead>
<tr>
<th>Measures</th>
<th>Sham</th>
<th>Pair-fed</th>
<th>Uremic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N = 32</td>
<td>N = 38</td>
<td>N = 39</td>
</tr>
<tr>
<td>Plasma creatinine</td>
<td>0.37 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.39 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.03 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>mg/dL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urea nitrogen</td>
<td>10.6 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.3 ± 0.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>36.7 ± 2.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>mg/dL</td>
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</table>

Animals were in the proestrous stage of the estrous cycle and underwent blood sampling during the presumptive luteinizing hormone (LH) surge. Data are expressed as the mean ± SEM. Values in the same row with different superscripts are significantly different by ANOVA followed by Duncan’s multiple range test (P < 0.05).

RESULTS

By 14 days after second-stage nephrectomy, the animals were assumed to be chronically renal insufficient. This condition was verified by the values for plasma creatinine and urea nitrogen, as shown in Table 1.

Of the 146 animals received for these studies, 111 (76%) exhibited four-day estrous cycles at the beginning of the experiments. Examination of the influence of the surgeries on the cycles showed that 27 of 31 (87%) sham animals continued to cycle normally after surgery. Of the pair-fed and uremic animals, 26 of 34 (76%) and 33 of 46 (72%), respectively, continued to cycle normally after surgery.

During the first four repetitions of the experiment, 83 animals were successfully sampled throughout the evening of proestrus, and the samples were assayed by RIA. The temporal dynamics of the preovulatory LH surge are shown in Figure 1. The surges in the sham and pair-fed animals were comparable, while the uremic animals showed an apparently lower level of release. To express the LH surges in values that could be more directly compared among the groups, the approximate “mass” of each surge was calculated. For each animal, a baseline was established, using the initial and final LH values. The approximate mass was calculated as the area under the curve, above baseline. These data are shown in Figure 2. The mean value for the uremic animals (15.6 ± 4.2 ng/mL/surge, N = 28) was significantly lower (P < 0.05) than that for either the sham (32.4 ± 4.6 ng/mL/surge, N = 24) or the pair-fed (26.8 ± 3.6 ng/mL/surge, N = 31) animals. These data include all of the...
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Observation of the LH mass data shows that LH was not significantly suppressed in uremic animals after brevital anesthesia. Ovulation, however, was clearly suppressed in uremic animals after brevital anesthesia. The aggregate data, however, showed an overall suppression of LH release. Ovarian weight was significantly different among experimental groups normalized to total body weight. Ovaries from uremic animals ($N = 54$) were larger (17.9 ± 0.5 mg/100 g body weight, $P < 0.05$) than those of either the sham (16.5 ± 0.4 mg/100 g body weight, $N = 64$) or the pair-fed (15.6 ± 0.3 mg/100 g body weight, $N = 68$) groups.

**DISCUSSION**

Uremic women typically present with clinically evident loss of regular menstrual cyclicity. More than 90% of premenopausal patients on dialysis lack regular menses, and approximately 40% are frankly amenorrheic [13, 20]. In the experimental uremia model studied here, elevations in plasma creatinine and urea nitrogen were relatively mild rather than end stage. Plasma creatinine was approximately 2.7-fold higher than control, and plasma urea nitrogen was about 3.4-fold higher than control. These values would translate into human values of approximately 3.2 mg/dL for creatinine and 70 mg/dL for urea nitrogen. This degree of uremia did not cause a specific deficit in estrous cyclicity in the female rats, that is, uremic and pair-fed animals manifested equivalent cyclicity. Thus, the alterations evident in this uremic model might mirror anticipated deficits in reproductive function that occur during relatively early stages of renal insufficiency, as opposed to those that might be expected to exist during dialysis. In this respect, the 5/6 nephrectomized rat might be suitable for determining initial disturbances in the reproductive axis, which eventually result in the loss of ovarian cyclicity.

We have observed that female rats ovulate as many as 18 ova or as few as 1 ovum per estrous cycle. The mean number of ova per oviduct in the sham group was approximately six, whereas in uremic rats, that continued to cycle normally, this number fell by more than 50%. Pair-fed animals did not show this decrement. The suppressive effect of uremia was independent of the type of anesthesia used preoperatively. Specifically, brevital showed the same inhibition of ova production (in comparison with the sham animals) as did animals given ether. The lack of suppression of the preovulatory LH surge in uremic animals after brevital anesthesia, however, might point to an inhibitory hypothalamic factor in uremia, the relief of which (by brevital) allowed LH release.

Another unexpected facet of our observations was the greater weight of the ovaries (normalized to total body weight) of uremic animals compared with sham or pair-

![Diagram](image-url)
Table 2. Relationship of luteinizing hormone (LH) surges and ovulation to the anesthetic used for nephrectomy (at least 14 days before sampling) and catheter implantation (the day before blood sampling)

<table>
<thead>
<tr>
<th>Repetition</th>
<th>Anesthesia</th>
<th>LH mass ng/mL/surge (N)</th>
<th>Ova per oviduct (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sham</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Ether</td>
<td>17.8 ± 10.1 (5)</td>
<td>N/A</td>
</tr>
<tr>
<td>2</td>
<td>Brevital</td>
<td>26.2 ± 5.2 (8)</td>
<td>6.1 ± 0.8 (16)</td>
</tr>
<tr>
<td>3</td>
<td>Ether</td>
<td>31.9 ± 6.2 (4)</td>
<td>6.0 ± 1.1 (8)</td>
</tr>
<tr>
<td>4</td>
<td>Ether</td>
<td>50.1 ± 9.9 (7)</td>
<td>5.5 ± 0.8 (14)</td>
</tr>
<tr>
<td>5</td>
<td>Ether IRMA</td>
<td>5.9 ± 0.9 (16) BioAsay</td>
<td></td>
</tr>
<tr>
<td>Aggregate</td>
<td></td>
<td>32.4 ± 4.6 (24)</td>
<td>5.9 ± 0.9 (54)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Repetition</th>
<th>Anesthesia</th>
<th>LH mass ng/mL/surge (N)</th>
<th>Ova per oviduct (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pair-fed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Ether</td>
<td>18.6 ± 7.7 (6)</td>
<td>N/A</td>
</tr>
<tr>
<td>2</td>
<td>Brevital</td>
<td>28.9 ± 11.4 (5)</td>
<td>3.1 ± 1.2 (10)</td>
</tr>
<tr>
<td>3</td>
<td>Ether</td>
<td>26.1 ± 5.6 (14)</td>
<td>5.7 ± 0.5 (28)</td>
</tr>
<tr>
<td>4</td>
<td>Ether</td>
<td>24.8 ± 6.6 (12)</td>
<td>5.3 ± 0.8 (5)</td>
</tr>
<tr>
<td>5</td>
<td>Ether IRMA</td>
<td>4.5 ± 0.9 (12) BioAsay</td>
<td></td>
</tr>
<tr>
<td>Aggregate</td>
<td></td>
<td>26.8 ± 3.6 (31)</td>
<td>5.0 ± 0.4 (64)</td>
</tr>
</tbody>
</table>

Blood samples were taken while the animals were awake and freely behaving. Use of ether for surgical anesthesia resulted in the specific suppression of the preovulatory LH surge in uremic animals (repetitions 1 and 3–5), while use of brevital (repetition 2) did not. Suppression of ovulation in uremic animals, compared to sham animals, was maintained without reference to the type of surgical anesthesia. “Aggregate” values include all data, without respect to anesthesia.

Another significant finding in premenopausal women with end-stage renal failure undergoing hemodialysis is that LH secretion, albeit somewhat pulsatile, fails to culminate in effective ovulation [13]. The uremic rats in the present study showed a diminutive surge of LH wherein the mean plasma LH concentration rose from 2.1 ± 0.3 ng/mL at 14.30 hours to 7.3 ± 1.6 ng/mL at 19.30 hours. The magnitude of this LH rise was significantly attenuated compared with that observed in either the sham (11.6 ± 1.8 ng/mL at 19.30 hours) or the pair-fed females (10.3 ± 1.5 ng/mL at 18.30 hours). These data allow the speculation that an early event in the pathway of reproductive decline in uremia might entail a progressive suppression of the preovulatory LH surge. In more severe uremia, the LH surge may fail completely. In this respect, the hypogonadism of uremia in the female might actually be quite conspicuous, although the underlying mechanisms remain somewhat obscure.

ACKNOWLEDGMENTS

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Fig. 3. Influence of uremia on ovulation rate. Values are expressed as the mean ± SEM number of ova per oviduct. The numbers of animals per group were 54 ovaries for sham, 64 ovaries for pair-fed, and 68 ovaries for uremic. Bars with different superscripts are significantly different (P < 0.05).

fed controls. The underlying cause of the increased ovarian size in uremic animals remains to be determined. One consideration is that prolactin might be elevated in uremic female rats and cause hypertrophy of the ovaries by maintenance of corpora lutea. Prolactin is known to be luteotropic in the rat, albeit deleterious to reproductive cyclicity, putatively via its hypothalamic actions [21]. In this regard, Lim, Kathpalia and Frohman have reported the occurrence of hyperprolactinemia in a significant number of uremic women [14].
REFERENCES


