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GAVIN PUBLISHERS

Journal of Urology and Renal Diseases

Levin RM, et al. J Urol Ren Dis: JURD-1125.

DOI: 10.29011/2575-7903.001125

Review Article

Cyclical Estrogen as a Major Etiology of Interstitial Cystitis: A Theory and Review

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Citation: Levin RM, Schuler C, Leggett RE (2018) Cyclical Estrogen as a Major Etiology of Interstitial Cystitis: A Theory and Review. J Urol Ren Dis: JURD-1125. DOI: 10.29011/2575-7903.001125

Received Date: 20 September, 2018; **Accepted Date:** 05 October, 2018; **Published Date:** 10 October, 2018

Abstract

Interstitial Cystitis / Pelvic Bladder Syndrome (IC/PBS) is a lower urinary tract dysfunction characterized by urgency, frequency, and pain upon bladder distension. Although there is no widely accepted etiology of IC, most researchers and clinicians believe it involves the breakdown of the mucosal permeability barrier and the penetration of solutes from the urine into the bladder mucosa and muscle, causing sensory neuro-inflammation and other cellular and sub-cellular pathologies resulting in IC/PBS. Our hypothesis is: Cyclical Estrogen is a Major Etiological factor of Interstitial Cystitis / Pelvic Bladder Syndrome: The menstrual cycle is characterized by cyclical changes in circulating estrogen. We completed two rabbit studies in which circulating estrogen was cycled between low estrogen (following ovariectomy) and high estrogen (following implantation of slow-release estrogen tablet). Circulating estrogen concentrations were cycled at two-week intervals (mimicking the menstrual cycle) for 4 complete cycles; ending either after a low estrogen period or after a high estrogen period. These rabbit studies on the effect of cyclical estrogen demonstrate that during low estrogen periods, blood flow, PO2, and blood vessel density decrease; mucosa thickness decreases, mucosal and smooth muscle hypoxia increase; free radical generation and oxidative stress increase; and the volume fraction of smooth muscle / collagen decreases. During high estrogen periods all responses to low estrogen return back to or toward normal. This time frame would make it very likely that these same changes occur in women during the normal menstrual cycle; which provides direct support of our hypothesis. Additional support comes from several clinical papers that show: 1) Estrogen variations during the menstrual cycle significantly affect bladder pain in IC/PBS patients. 2) On a cellular level, estrogen directly modulates pain mechanisms. And 3) if one suppresses circulating estrogen, or increases circulating estrogen continually, IC/PBS pain symptoms are relieved but return when cyclical estrogen is restored

Although the above studies provide direct support that cyclical estrogen has marked cyclical effects on bladder smooth muscle and mucosal blood flow, nerve density, oxidative stress, hypoxia, and structure (including hypertrophy, hyperplasia, apoptosis, and smooth muscle / collagen ratios). At this time there is no direct evidence that the pain associated with IC/PBS is directly related to either low or high estrogen periods.

Keywords: Bladder; Estrogen; Interstitial Cystitis; Ischemia; Oxidative Stress; Painful Bladder Syndrom; Rabbits; Reperfusion

Introduction

Interstitial Cystitis (IC) and its co-name Painful Bladder Syndrome (PBS) is a Lower Urinary Tract Dysfunction (LUTD) characterized primarily by urgency, frequency, and pain upon bladder distension (filling) [1-3]. The large majority of patients are women post puberty and prior to menopause; and thus is not specifically related to ageing [4,5]. Although there is no widely accepted etiology of IC, and treatments are generally based strictly on alleviating the symptoms [6-9], most researchers and clinicians believe that whatever the cause, it involves the breakdown of the mucosal permeability barrier and the penetration of solutes from the urine into the bladder mucosa and muscle that cause sensory neuro-inflammation and other cellular and sub-cellular pathologies resulting in the general symptoms of IC/PBS [9-11]. In current reviews of IC/PBS [1,12] the authors agree that at this time there is no unified theory or etiology of IC/PBS, and provide a review of a variety of possible etiologies including: Glycosaminoglycan theory,

Microbial/infection theory, Mast cell theory, Neuroendocrine theory, Neural upregulation - Sensory nerve upregulation theory. In virtually all theories of pathophysiology, inflammation plays one a pivotal role in the onset and progression of IC/PBS [1]. Because of the diffuse nature of the pathophysiology, several other lower urinary tract dysfunctions have been co-linked with IC/PBS including endometriosis; which occurs when the endometrial lining of the uterus begins to expand outside the uterus to other pelvic organs. Which also results in pelvic pain [13].

Although not listed as an etiological factor in IC/PBS, there is an excellent discussion of the direct involvement of estrogen in the progression of IC/PBS [1]. IC / BPS symptoms can be associated with changes in local (lower urinary tract) estrogen. Several studies suggest that estrogen replacement should be considered in the treatment of this condition [14-16]. Other investigators have similarly proposed an estrogen-based onset IC/PBS symptom. These authors also propose a possible link between menstrual cycle and IC/PBS symptoms, including pain [17-19]. In one clinical study [20] women that were affected by genital prolapse were enrolled in this study. At entry, participants were randomized into two equal groups. The women in group 1 were treated daily with vaginal gel containing 50μg estriol for 12 weeks prior to surgery. The women in group 2 did not receive any estrogen treatment. Women were evaluated after this 12-week period (prior to surgery). One week after surgical treatment, the women in group 1 were divided into 2 subgroups: Group 1A continued estriol vaginal gel for an additional 12 weeks, and group 1B discontinuing the estrogen treatment. The second follow-up examination was performed following the end of the study. The results demonstrated that all aspects of vaginal health improved in group 1 both before and following surgery, while group 1B (stopping estradiol following surgery) significantly reduced the improved vaginal health demonstrated in group 1A. Although not directly related to IC / BPS, these studies are consistent with our studies showing the beneficial effects of continuous estrogen administered to ovariectomized animals [21-26]. In addition to the human studies described above, there is also evidence in animal models that changes in circulating estrogen may be related to IC-like symptoms [12,27,28].

Role of the Mucosa in Bladder Function and Dysfunction

The bladder mucosa is intimately associated with lower urinary tract function [29-31]. The mucosal surface is the first line of defense against bladder infection, presenting a non-adherent surface toward most strains of bacteria. In addition, the mucosa (glycosaminoglycan coating) provides a relatively impermeable barrier to the movement of solutes from the urine into the underlying structures [32-39]. As stated above, a substantial body of evidence now exists which indicates that Interstitial Cystitis (IC) is related to a breakdown of mucosal integrity [32-40]. The symptoms of IC/PBS involve an increase in urothelial permeability and the penetration of ions and other caustic substances in the urine into the mucosa and submucosa. The best direct evidence is Dr. Parsons' demonstration that permeability to urea is more than 5-fold greater in IC/PBS patients than in normal women, and that more than 70% of IC/PBS patients respond to intravesical KCl administration with significant discomfort, whereas virtually no control women responded [32,33,40].

Relationship of Blood Flow and Cellular Metabolism to IC/BPS

Beneath the urothelium, and sometimes protruding through the basal laminae, is a rich plexus of capillaries, the suburothelial capillary plexus [41-43]. The glomerulations, which are characteristic of mucosa in hydrodistended bladders of patients with IC, probably represent ruptured elements of this plexus. Since the presence of distension-generated glomerulations is perhaps the one characteristic of IC/PBS that is agreed upon by virtually all urologists involved in the treatment of IC, the importance of this capillary system (and local blood delivery to the mucosa) cannot be overemphasized [44-48]. Our current studies, and those of others, show that urothelial function is severely compromised by both ischemia and hypoxia [29,38,39,49,50]. Changing the aeration of an *in vitro* whole bladder preparation from oxygen to nitrogen induced a hypoxic condition. As soon as the bath's oxygen tension fell below 20%, mucosal permeability to dye increased proportionally to the level and duration of hypoxia, even at low intravesical volumes [51]. Normal bladder distension in the presence of mild hypoxia / ischemia promoted mucosal dysfunctions resulting in increased permeability [51].

One additional fact is that the bladder mucosa has a significantly higher metabolic rate than the underlying smooth muscle, and a significantly lower concentration of high energy phosphates [29,30]. This makes the mucosa very sensitive to hypoxia and ischemia. These studies demonstrate the sensitivity of mucosal permeability to reduced oxygen tension and reduced blood flow. Recent evidence indicates that there is a significantly reduced local blood flow to the mucosa in association with interstitial cystitis. Using laser doppler flowmetry, Irwin and Galloway presented excellent evidence that during distension, perfusion was significantly reduced in women with interstitial cystitis; blood flow of non-IC/PBS patients were not affected [52,53]. It is well documented that bladder distension can induce decreased blood flow in proportion to the distension [54-56]. Clinically, it is clear that the pain and urgency experienced by IC patients are induced by distension and relieved by voiding; these events are consistent with the hypothesis that distension induces a relative mucosal ischemia, increasing permeability and sensory-pain activation (IC/PBS symptoms). Both the relative ischemia and symptomology

(pain) are relieved by bladder emptying.

Estrogen effects on tissue oxygen tension and blood flow

Blood flow and tissue oxygen tension to the lower urinary tract, as well as to the vagina and uterus, can be affected significantly by alterations in estrogen [57-60]. Estrogen has been shown to increase blood flow to the bladder and urethra whereas low estrogen reduces blood flow below normal and induces hypoxia / ischemia even in un-distended bladders [25,59,60]. The demonstration that blood flow can be modulated by alterations in estrogen, together with the finding by Irwin and Galloway that blood perfusion is reduced during distension in IC/PBS patients [52], support the importance of reduced blood flow in this gender-related disorder. And one major target for ischemia and hypoxia is nerve membranes [31,61]. Compliance is a function of bladder wall tension. Decreased compliance relates to an increase in bladder wall tension; and can result from either increased collagen composition (decreased smooth muscle / collagen ratio), decreased lipid content of the bladder membranes, or increased concentration of intracellular free calcium, or a combination. Compliance relates to the change in bladder pressure per ml volume during a cystometry. Ovariectomy (Ovx) decreases compliance (makes the bladder stiffer) both as the bladder begins to fill and at micturition. Estrogen completely reverses the effect of Ovx on compliance [26,59,62]. In recent studies in our lab we have clearly demonstrated that ovariectomy results in significant decreases in both tissue oxygen tension and blood flow to the bladder mucosa and to a lesser extent to the detrusor smooth muscle, which is correlated with mucosal hypoxia, apoptosis, atrophy, increased oxidative stress, and increased permeability. Estrogen administration to ovariectomized rabbits resulted in the relief of hypoxia, mucosal hyperplasia and restoration of the mucosal permeability barrier [25,59,60]. In chronic studies [25,26,59,63], female rabbits were divided into three groups of 6 rabbits each. The rabbits in group 1 received ovariectomies, group 2 received sham operations, and group 3 received ovariectomies + estrogen therapy at 1mg/kg/week via Alzet pump (Ovx +E) beginning 1 week following ovariectomy and continuing for 5 additional weeks.

The results can be summarized as follows: Ovx resulted in: 1) a rapid and substantial decrease in plasma estrogen and progesterone concentrations; 2) Significant decreases in tissue PO_2 in both the muscle and mucosal compartments; 3) a significant decrease in bladder compliance; 4) significantly decreased blood flow to the bladder mucosa and a smaller but statistically significantly decreased blood flow to the bladder smooth muscle; 5) thinning of the bladder mucosa; 6) generalized hypoxia of the mucosa, submucosal elements, and endothelial elements in the smooth muscle; and 7) significantly increased mucosal permeability. Most dramatically Ovx resulted in a rapid and prolonged increase in oxidation products of reactive oxygen free radicals (ROS).

ROS products include the generation of reactive protein carbonyl groups, which can be quantitated by the derivatisation of the carbonyl group with 2,4-Dinitrophenylhydrazine (DNPH), which then leads to formation of a stable dinitrophenyl (DNP). DNP can then be quantitated by Western Blot. Reactive nitrogen free radicals (RNS) is quantitated by the analysis for Nitrotyrosine (NT), which is also quantitated by Western Blot analysis. A third oxidative stress biomarker is the lipid peroxidation product Malondialdehyde (MDA) which is quantitated biochemically. The detailed methodologies for analysis of oxidative stress biomarkers can be found in the references given [64-66]. Ovx also resulted in significant decreases in the *in vitro* contractile responses to field stimulation, carbachol, and KCl [21,25,26,63,67-70].

Estrogen administration to Ovx rabbits resulted in: 1) Significant increases in tissue PO_2 and blood flow in both the muscle and mucosal compartments back to control levels; 2) a significant increase in bladder compliance; 3) thickening of the bladder mucosa primarily via hyperplasia; 4) relief from hypoxia of the mucosa, submucosal elements, and endothelial elements in the smooth muscle; 5) a significant decrease in mucosal permeability to pre-Ovx levels. Most dramatically estrogen resulted in a rapid but incomplete decrease in some oxidation products. In addition, the *in vitro* contractile responses returned to control levels [21,25,26,63,67-70].

The Rabbit as an Excellent Model to Study Lower Urinary Tract Function

First, the rabbit urinates only 4-6 times per day which is similar to humans. Micturition volume and frequency can be accurately quantitated using metabolic cages and continual collection and recording (via computer interface). Any increase in frequency or volume of micturition would be very clear with the use of rabbits as opposed to what might be seen when using mice, rats or guinea pigs. A significant increase in micturition frequency and decrease in micturition volume would be directly related to urgency and frequency.

Second, cystometry can be performed through the urethra and thus is minimally invasive and does not require any surgical methods. Cystometry can be performed under mild sedation (urethane) or under complete anesthesia (pentobarbital). Under sedation, cystometry will quantitate micturition pressure, volume at micturition, compliance, and the presence of overactive bladder dysfunction (frequency and pressure) which is not present in normal female rabbits. Under anesthesia, cystometry will also quantitate micturition pressure, volume at micturition, and compliance. Overactive bladder dysfunction will be completely inhibited. For normal rabbits, cystometries under mild sedation or anesthesia are similar. If an IC-like condition exists, i.e. pain upon distension, the micturition pressure is similar, volume at micturition is significantly decreased and compliance is significantly decreased under mild sedation compared to anesthesia. Overactive bladder dysfunction may be present under mild sedation but not under anesthesia

Third, urethral Pressure Profiles (OPP) can be measured by placing a UPP catheter in the bladder and removing it slowly while continually measuring the pressure as the UPP exits through the urethra. Fourth, mucosal permeability can be evaluated in vivo by placing 20 ml of 1% Trypan blue in the empty bladder for 30 minutes and then washing the bladder thoroughly with saline. Trypan blue will not attach to or penetrate normal bladders but will attach to and penetrate bladders with damaged mucosa [50,59,71,72]. The level of permeability can be evaluated using a pediatric cystoscope which is placed in the bladder via the urethra and the color of the mucosa can be quantitated using video and photographic analytical software. We use Trypan blue rather than indigo carmine because within one month Trypan blue will be eliminated from the tissue whereas the indigo carmine stain is permanent [50]. Thus, all aspects of bladder function can be easily evaluated and quantitated without the need for invasive methodologies, which allows for longitudinal studies using the same groups of rabbits.

Our hypothesis is: Cyclical Estrogen is a Major Etiological factor of Interstitial Cystitis

The menstrual cycle is characterized by cyclical changes in circulating estrogen. (Figure 1) is a schematic of the menstrual cycle. Prior to ovulation there is rapid and significant increase in circulating estrogen. Prior to ovulation is the follicular phase which is characterized by uteral hypertrophy and hyperplasia as well as bladder mucosal hypertrophy and hyperplasia [73-76]. Following ovulation is the Luteral phase characterized by menstruation and uteral sloughing, and mucosal apoptosis and regression. Although the uterus is the primary target of estrogen, showing significant cellular proliferation and enlarging as estrogen increased, and undergoes apoptosis, thinning, and sloughing during low estrogen periods (menstruation). The bladder is a secondary target, in that the bladder mucosa undergoes hyperplasia and thickening during high estrogen periods, and apoptosis and thinning during low estrogen periods [62,77]. The result of these cycles of high estrogen followed by low estrogen with the accompanying changes in blood flow and ischemia and subsequent cycling of oxidative stress induces the characteristic urgency, frequency, and pain upon bladder distension which are characteristic of IC/PBS [62,77]. This theory would also explain why IC/PBS generally does not affect girls prior to puberty, and often disappears following menopause.

One important question that needs to be discussed is: Since menstruation affects most women, why isn't IC/PBS more prevalent, or occurs only during periods of low estrogen. The pain upon distension is believed to be due primarily to stretch-activated pain afferents with the participation of inflammation

within the nerves [78-80]. In the same way that not all diabetics develop diabetic neuropathy; and not all smokers develop lung cancer; there are mitigating factors that make specific individuals sensitive to the development of IC/PBS, diabetic neuropathy, and lung cancer. In an interesting meta-analysis study of IC/PBS and birth control pills, the major finding was that women taking oral contraceptives have a significantly increased incidence of IC/PBS than women that do not take oral contraceptives [81]. Since the way oral contraceptives are given, a woman takes one pill a day for 21 days thus maintaining estrogen at a high constant level, and then during the 7 days off of the pill, estrogen falls precitously and thus enters a low estrogen state. Alternately, when women start the next cycle of oral contraceptives they shift from a low estrogen period to a high estrogen period in a very short time that induces a period of "reperfusion" which results in an acute period of significant free radical generation and oxidative stress [82-84], The fact that women on oral contraceptives have a significantly higher incidence than women who do not take the pill provides support for our theory [81]. Similar correlations between estrogen and IC/PBS have also been reported [85].

It should be stressed that these studies showed an increased incidence of IC/PBS and certainly not that all persons treated with estrogen developed IC/PBS. One additional factor that should be discussed is that in our studies, low estrogen (ovariectomy) results in significant ischemia and oxidative stress, and prolonged estrogen administration relieves the ischemia and oxidative stress. The initial effects of changing to high estrogen stimulates "Reperfusion" which results in an acute period of significant free radical generation and oxidative stress [82-84], which correlates very well with the study on oral contraceptives discussed above. Although not as common, IC/PBS also occurs in men. It is not sure if the etiology of IC/PBS in men is similar to that in women; there are some similarities that correlate the two diseases. As discussed above, during high estrogen periods blood flow increases and oxidative stress decreases; whereas during low estrogen periods oxidative stress significantly increases correlated with significantly decreased blood flow. Although men do not have cyclical changes in circulating hormones; they do have cyclical changes in oxidative stress and blood flow in relation to bladder filling and emptying [84,86-88]. As the bladder fills, intrawall compression increases reducing blood flow to the entire bladder. The result is hypoxia and the generation of free radicals (oxidative stress). When the bladder voids, oxygen levels return very quickly inducing reperfusion with the added generation of oxidative free radicals [88,89]. This sequence is much more severe in men than women because of the significantly greater urethral pressure in men due to the encircling prostate. Since men don't have the conflicting influence of cyclical hormones, the incidence of IC/PBS in men is significantly lower than in women. The common factor is cyclical oxidative stress. The following study provides direct evidence for our hypothesis: All rabbit experiments were approved by the Stratton VA Medical Center Institutional Animal Use and Care; and Research and Development Committees.

Forty adult female NZW rabbits were divided into 5 groups of 8 rabbits each. Group 1 were control rabbits (which received sham operations). Groups 2 through 5 received bilateral ovariectomies by standard sterile technique [25, 26]. Group 2 (OVX) received no estradiol. Groups 3 (OVX + E) received 17-ß estradiol as a slow release tablet (Innovative Research of America, Sarasota, FL, USA) at a dose of 0.1 mg/kg/day pladed in the subscapular region of the rabbit's neck. The dose remained constant and in place for the duration of the study. Group 4 had an estrodiol tablet implanted during the surgery. Group 4 rabbits had their estradiol tablets removed after two weeks on estradiol for a two-week period and then replaced for an additional two weeks; this cycling continued for 4 complete cycles. Group 4 rabbits ended the study after two weeks off of estradiol (Cyclic – E). Group 5 had an estradiol tablet implanted two weeks following ovariectomy. Group 5 rabbits had their estradiol tablets removed after two weeks on estradiol for a two-week period and then replaced for an additional two weeks; this cycling continued for 4 complete cycles. Group 5 rabbits ended the study after two weeks on estradiol (Cyclic + E). Thus, we had a control group, and Ovx only group, an Ovx + Estrogen group; and two cyclical estrogen groups, one that ended at the end of a low estrogen period (Cyclic – E) and one that ended on a high estrogen period (Cyclic + E).

(Figures 1-4) are adapted from our figures published previously [62,77]. (Figure 1) shows changes in bladder compliance and volume fraction of smooth muscle for the 5 groups. Values were normalized to control = 100 for statistical reasons. During all periods of low estrogen (Including the periods of low estrogen not shown in figure 1) there were significant decreases in compliance (increase in bladder stiffness). Similarly, during all periods of high estrogen there were significant increases in compliance (decrease in bladder stiffness). The volume-fraction of smooth muscle decreased during low estrogen periods and increased during high estrogen periods. This is directly related to the changes in bladder compliance, since the increase in stiffness observed during low estrogen periods (increased compliance) would be consistent with a decreased volume fraction of smooth muscle / collagen; while the increased compliance observed during high estrogen periods would be consistent with the increased smooth muscle / collagen ratio.

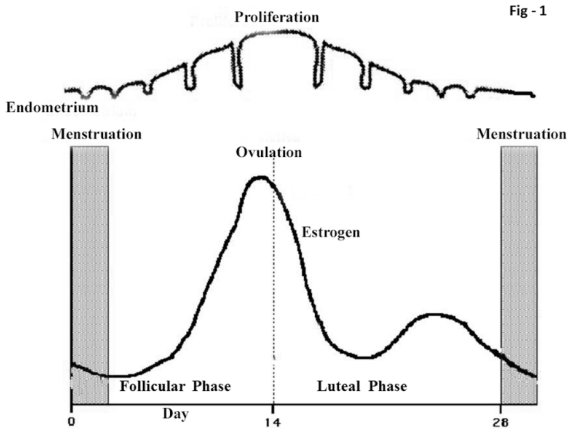

Figure 1: Schematic of the menstrual cycle showing the circulating estrogen, uteral hypertrophy, follicular and luteal phases, this figure is a modified free clip-art schematic

(Figure 2) shows the effect of cyclical estrogen on both in-vivo blood flow using laser Doppler technology and blood vessel density quantitated via immunohistochemistry. Values were normalized to control = 100 for statistical reasons. Both blood flow and blood vessel density showed that ovariectomy results in significant decreases in both studies. Estrogen administration completely reversed the effect of Ovx. Cycling estrogen showed the same results; ie decreased blood flow and blood vessel density during low estrogen periods and increased blood flow and blood vessel density during high estrogen periods. Although we expected these changes in blood flow, we did not expect the rapid changes in blood vessel density [24,62,77].

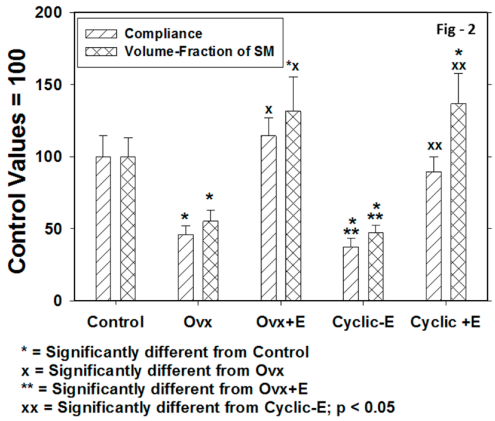

Figure 2: Effect of cyclic estrogen on compliance and volume fraction of smooth muscle: Each bar is the mean +/- SEM of 4 rabbits. Ovx = ovariectomy; Ovx + E = ovariectomy plus continuous estrogen; Cyclic – E = cyclical estrogen ending at the end of a low estrogen period. Cyclic + E = cyclical estrogen ending at the end of a high estrogen period. * = Significantly different from control; x = significantly different.

(Figure 3) shows that the oxidative stress level of smooth muscle of the bladder body is also sensitive to cyclical estrogen. The concentration of free radicals increased during low estrogen periods and decreased during high estrogen periods.

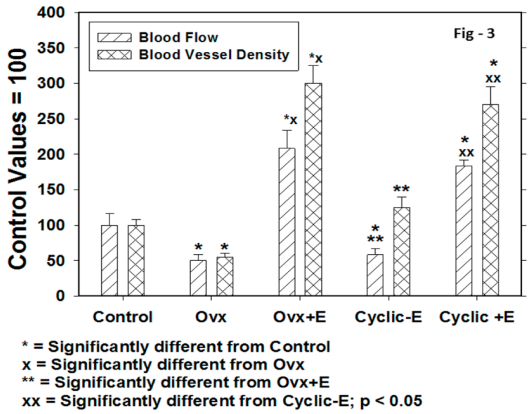

Figure 3: Effect of cyclic estrogen on blood flow and blood vessel density: Each bar is the mean +/- SEM of 4 rabbits. * = Significantly different from control; x = significantly different from Ovx; ** = significantly different from Ovx + E; xx = significantly different from Cyclic – E; $p < 0.05$.

(Figure 4) displays the contractile responses to Field Stimulation (32 Hz), carbachol (20 µM), and KCl (120 mM). The contractile responses to all forms of stimulation were reduced during low estrogen periods and increased during high estrogen periods. This would be consistent with the cyclic alterations in smooth muscle / collagen ratios described above.

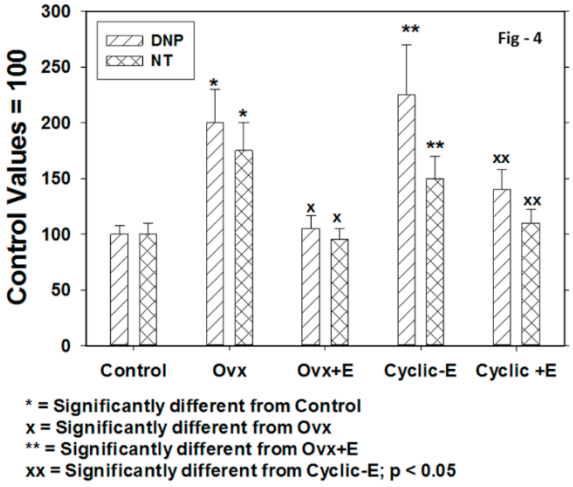

Figure 4: Effect of cyclic estrogen on the Oxidative Stress Products: Each bar is the mean +/- SEM of 4 rabbits. * = Significantly different from control; x = significantly different from Ovx; ** = significantly different from Ovx + E; xx = significantly different from Cyclic – E; $p < 0.05$.

(Figure 5) Although these cyclic alterations in blood flow, blood vessel density, free radical generation – oxidative stress, compliance, and contractile responses are all consistent with urological changes with the menstrual cycle of women, it does not explain the major symptom of pain upon bladder distension.

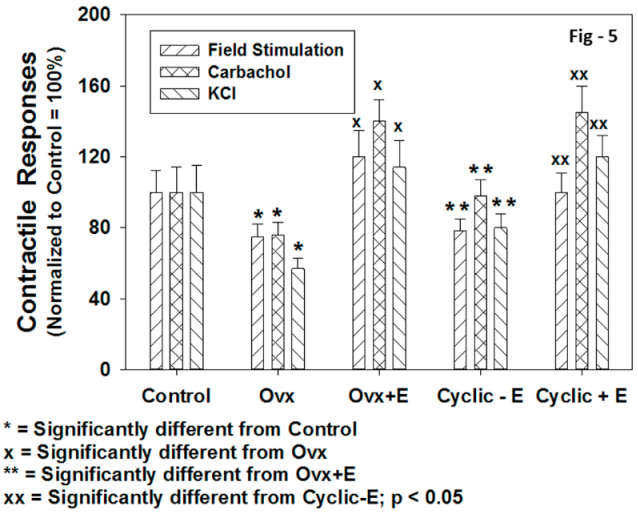

Figure 5: Effect of cyclic estrogen on *in vitro* contractile responses: Each bar is the mean +/- SEM of 4 rabbits. * = Significantly different from control; x = significantly different from Ovx; ** = significantly different from Ovx + E; xx = significantly different from Cyclic – E; $p < 0.05$.

The above studies clearly demonstrate a cyclical nature in blood flow, free radical generation, and oxidative stress with changes in estrogen levels, which we link to IC/PBS; although we are not predicting that the pain associated with bladder filling is due to the blood flow and oxidative stress associated with the low estrogen period. When blood flow increases rapidly as occurs when estrogen is administered, there is a period of reperfusion which is characterized by high levels of free radical generation and oxidative stress. Thus, oxidative stress (free radical generation) occurs during the two-week period of low estrogen, and for a short period of time when estrogen is administered, mediating a rapid increase in blood flow (reperfusion) resulting in a significant spike of oxidative stress. In an interesting study of the effects of in-vivo ischemia / reperfusion in rabbits. Rabbits were made ischemic for 2 hours and then reperfused for 2 hours, 7 days, and 14 days [82]. These studies clearly demonstrated that within two hours after the start of reperfusion there were significant decreases in nerve densities in the detrusor muscle using both immunohistochemistry to visualize neurofilament density and quantitate nerve density. There were also significant decreases in choline acetyltransferase (the enzyme that synthesizes the neurotransmitter acetylcholine) and significantly increased levels of apoptosis (programmed cell death). These statistical comparisons were made between the two-hour reperfusion group, vs the ischemia alone and the control groups. Similar experiments in the rat also demonstrated that reperfusion injury is more severe than injury by ischemia alone [83].

Thus, if the pain upon distension is mediated by this spike in oxidative stress that occurs during reperfusion, it would occur during the high estrogen period and not the low estrogen period. It is our second hypothesis that the prolonged estrogen cycling that occurs during the menstrual cycle that in some specifically sensitive women develop progressive sensory nerve damage based on the cyclical hypoxia and oxidative stress that occurs, which in turn mediates bladder pain caused by the stretching of the damaged sensory nerves. As presented above, this can be induced both during the low estrogen period, and during the initial phases of the high estrogen period. This can be investigated by extending the duration of the estrogen cycling described in these studies. One of the most interesting facts relates to the speed that these changes occur (two-week intervals). This time frame would make it very likely that these same changes occur in females during the normal menstrual cycle; which provides direct support of our hypothesis. In an excellent study by T. Powell-Boone, et al. [19]. 7 Female participants diagnosed with IC/PBS and 8 healthy controls studied participated. The participants completed daily diaries related to bladder pain, and daily micturition frequency was recorded [19]. Cystometrograms were performed two times during the study, during the luteal and follicular phases. IC/PBS patients had higher pain scores and frequency than controls throughout the entire menstrual cycle. However, pain scores were highest in the perimenstrual period in subjects with IC and surprisingly also in the controls, although at an extremely lower score. This correlates at times with high circulating estrogen. Micturition frequency was significantly higher for the IC/PBS patients than controls at all times throughout the cycle. Bladder pain was observed at lower intravesical volume and pressure during the follicular period than during the luteal period in IC/PBS patients [19].

Interestingly similar findings were found in the controls, although at significantly higher volumes. These findings are consistent with the idea that bladder pain in IC/PBS is strongest at the perimenstrual time period in both IC/PBS and control participants. This study indicates that the pain occurs during the high-estrogen portion of the menstrual cycle. Published studies by ourselves and others have clearly demonstrated that rapid reperfusion following ischemia (low estrogen) result in significant generation of free radicals and oxidative stress [82-84]. Thus, oxidative stress occurs during both the periods of low estrogen and during the initial phase of the reperfusion period (high estrogen period). It is clear from clinical studies that the acute symptoms of IC/PBS can occur anytime during the estrogen cycling. In a second study not limited to IC patients, pain perception during the menstrual cycle in women diagnosed with functional syndromes such as IC/PBS and

Irritable Bowel Syndrome (IBS) suggests strongly that modulation of estrogen significantly afferent nociceptors located in the afferent primary sensory neurons [90]. This study also supports our theory and is consistent with our data. One additional interesting study involved treating IC/PBS patients with both leuprolide acetate and oral contraceptive pills Luprolide is a synthetic gonadotropin-releasing hormone used to treat symptoms of endometriosis. It works by suppressing the release of estrogen thus represents period of low estrogen. The oral contraception creates a chronic high estrogen period. Both treatments were effective in relieving the symptoms of IC/PBS until the treatment had stopped at which time the symptoms returned [91]. This study is consistent with our theory that it is the cyclical nature of the menstrual cycle that induces IC/PBS in susceptible women, and not the estrogen level itself.

Acknowledgement

This manuscript was funded in part by the Office of Research and Development Department of the Veterans Affairs, and by the Capital Region Medical Research Foundation.

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