Uterine Reactivity During The Estrous Cycle Phase of Experimentally-Induced Hyperurecemic Rats

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Abstract

Hyperuricemia is considered as an important risk factor of miscarriage and prematurity. This study aims to demonstrate if there is a direct effect of hyperuricemia on the spontaneous uterine contractions or uterine reactivity. Hyperuricemia was induced by 4 weeks of daily intraperitoneal (i.p.) injection of oxonic acid potassium salt (250mg/kg) in a group of 10 rats. Another group (10 rats) received oxonic acid potassium salt and allopurinol (150 mg/L drinking water) daily for 4 weeks. A control group (10 rats) was injected daily by 0.9% saline solution i.p. for 4 weeks. At the end of the experiment, systolic blood pressure was measured and serum uric acid (UA), serum urea, serum creatinine, serum malondialdehyde (MDA) and erythrocyte superoxide dismutase (SOD) activity were estimated. The uterine horns were taken for recording their reactivity to oxytocin and Ach. The data showed that hyperuricemia significantly increased serum UA, plasma MDA and systolic blood pressure and significantly decrease SOD activity, but insignificantly changed serum urea and creatinine and unexpectedly insignificantly changed the spontaneous uterine contractions and uterine reactivity to either of acetylcholine or oxytocin. Allopurinol treatment significantly decreased serum UA, plasma MDA and systolic blood pressure but insignificantly changed serum urea & creatinine, SOD activity, spontaneous uterine contractions and the uterine reactivity to either of Ach or oxytocin.

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INTRODUCTION

Hyperuricemia is defined as a serum uric acid (UA) concentration in excess of urate solubility, more than 7 mg/dl (420 μmol/L) in men or more than 6 mg/dl (360 μmol/L) in women [1]. UA is the end product of purine metabolism pathway in humans. In the majority of mammals, UA is further degraded to allantoin via the urate oxidase (uricase) enzyme in the liver. Allantoin is 5–10 times more soluble than UA, so it is freely excreted from the body in the urine [2]. Many factors contribute to hyperuricemia, including: genetics, insulin resistance, hypertension, renal insufficiency, obesity, diet, use of diuretics, and consumption of alcoholic beverages [3]. UA was considered a biologically inert substance, but then was found to have many biological properties that could be either beneficial or detrimental to humans [4]. Increased serum UA levels have been reported to be associated with hypertension [5,6], cardiovascular diseases [7,8], peripheral arterial disease [9], increased markers of inflammation [10], oxidative stress [11] and preeclamptic pregnancies [12]. Moreover, it was associated with increased risk of prematurity. The main pathophysiological mechanisms by which UA exerts these deleterious effects are endothelial dysfunction through impaired nitric oxide (NO) production and release, increased oxidative stress mainly through xanthine oxidase, low density lipoprotein (LDL) oxidation and lipid peroxidation), platelet activation, vascular smooth muscle cells proliferation and pro-inflammatory activity [13]. The risk of adverse maternal and fetal outcome increased with increasing concentration of UA in hypertensive pregnancy [14].

In this investigation we study the effect of oxonic acid induced hyperuricemia on oxidative stress parameters, some renal function parameters, blood pressure, spontaneous hypertension and uterine reactivity.

MATERIALS AND METHODS

Animals:

Thirty adult non-pregnant female albino rats of local strain weighing 140-180 grams were used in this study. Animals were fed with standard laboratory chow and water adlibitum and housed in animal house at Menoufia faculty of Medicine under artificial light/dark cycle of 12h. The animals were divided into 3 groups (10 each): **Control group**: rats were injected daily by 0.9% saline solution intraperitoneally (i.p.) for 4 weeks, **hyperuricemic group**: rats received i.p. injection of oxonic acid potassium salt dissolved in 0.9% saline solution at a dose of 250 mg/Kg body weight [15] daily for 4 weeks. **Hyperuricemic allopurinol-treated group**: rats received oxonic acid potassium salt (250mg/kg i.p.) and allopurinol (5mg/kg orally) [15] daily for 4 weeks to distinguish the effects of hyperuricemia from those of oxonic acid. Allopurinol is an inhibitor of xanthine oxidase, which is the enzyme responsible for uric acid synthesis [16]. At the end of the experiment, systolic blood pressure was measured using rat tail sphygmomanometer technique [17]. Vaginal smears were taken and examined under light microscope for detection of estrous phase. Then retro-orbital blood samples were collected for estimation of serum UA, serum urea, serum creatinine, plasma malondialdehyde (MDA) and erythrocyte SOD activity. Rats were then sacrificed by cervical

decapitation, and the uterine horns were taken for recording their reactivity to oxytocin and Ach.

Chemicals:

Chemicals used for preparation of Krebs-Hanseleit solution were purchased from Sigma (St Louis, Mo,U.S.A, oxonic acid potassium salt (Sigma-Aldrich Chemical Co. Steinheim, Germany), Allopurinol in form of zyloric tablets 100mg/tablet (GlaxoSmithkline S.A.E. El Salam City, Cairo, A.R.E.), Ach (El-Gomhoria Company, Egypt), Oxytocin in form of syntocinone ampoules 10 IU/ml = 20µg/ml (Novartis Pharma company-Switzerland). Kits used for estimation of serum urea and creatinine (El-Gomhoria Company, Egypt), & also Kits for estimation of serum MDA and erythrocyte SOD activity (Biodiagnostic Company, Egypt).

Measurement of systolic blood pressure: using the rat-tail sphygmomanometer method (Harvard apparatus Ltd, Adenberidge, England) where un-anaesthetized rats were placed in a plastic holder mounted on a thermostatically controlled warm plate. Averages of three reading were taken for each animal after it has been acclimatized to the environment. Systolic blood pressure was estimated from the recorded graph [17].

Blood sampling and biochemical analysis:

Blood samples were collected from the retro-orbital venous plexus, using a fine heparinized capillary tube introduced into the medial epicanthus of the rat's eye [18]. Two milliliters of blood were collected in a clean graduated centrifuge tube, left for clotting at room temperature in a water bath for 10 minutes, and then centrifuged at 3000 rotation per minute (r.p.m.) for 20 minutes. The supernatant serum was collected in a dry clean tube to estimate UA,

urea and creatinine. Also 0.5 mL of blood was collected from each animal using capillary tube in a vial containing EDTA as an anticoagulant then centrifuged at 4000 r.p.m. for 10 minutes, the plasma was used for estimation of plasma MDA and then erythrocyte lysate was prepared for estimation of erythrocyte super oxide dismutase activity.

Vaginal smear:

Vaginal secretion was collected with a plastic pipette filled with 10 µL of normal saline (NaCl 0.9%) by inserting the tip into the rat vagina, but not deeply. Vaginal fluid was placed on glass slides. One drop was collected with a clean tip from each rat. Unstained material was observed under a light microscope. Three types of cells could be recognized: round and nucleated ones are epithelial cells; irregular ones without nucleus are the cornified cells; and the little round ones are the leukocytes. The proportion among them was used for the determination of the estrous cycle phases [19].

Isolation of the uterus and recording of isotonic uterine contractility:

Rats were sacrificed, their abdomen were opened and the two uterine horns from each rat were exposed by pulling the intestine avoiding stretching of uterine smooth muscles. One horn from each rat was freed from its surrounding fat and mesenteric attachments. A uterine horn was transferred to a petri dish containing Krebs solution. The uterine horn was mounted in 40 ml organ path containing krebs solution. The lower end of the uterine horn was connected to a hook at the lower end of the tissue holder. The other end of the horn was connected via a thread to light isotonic lever. The contractions of the uterine horns were recorded on sheet paper on rotating

kymograph at a speed 1mm/sec. Preparation was suspended in Krebs solution, aerated with carbogen (95% O_2 and 5% CO_2) and maintained at 37 °C. The spontaneous uterine contractions were recorded then serial dilutions of Ach and oxytocin in cumulative pattern were added every 5 minutes. Each drug was tested separately. The amplitude and frequency of the spontaneous contractions were calculated before and after addition of the drugs. Since the uterine horns of estrous rats have their spontaneous contractions, the action of oxytocin or acetylcholine was expressed qualitatively in term of increasing rhythmicity or phasic contractions and/or tonic contraction of the tissue.

Statistical analysis:

The data were tabulated and analyzed by SPSS (statistical package for the social science software) using statistical package version 16 on IBM compatible computer. Quantitative data were expressed as mean $\pm$ standard deviation (X$\pm$ SD). The data from control and test groups were compared using an independent sample t-test. Probability value of less than 0.05 was considered as statistically significant (*P<0.05). "n" indicates the number of tested rats.

RESULTS

Oxidative stress and renal function changes:

Table 1 shows comparative data of serum UA, Urea & Creatinine, plasma MDA, SOD activity in all groups. In hyperuricemic group there was significant increase (P<0.01) in serum uric acid, plasma MDA and significant decrease in erythrocyte SOD levels when compared to the control group, while serum urea and creatinine show insignificant (P>0.05) change when compared to the control group. In hyperuricemic-allopurinol treated group there was significant decrease (P<0.01) in serum uric acid and plasma MDA levels when compared to the hyperuricemic group, while serum urea, creatinine and erythrocyte SOD show insignificant change (P>0.05) when compared to the hyperuricemic group.

Table (1): Oxidative stress and renal function changes in control, hyperuricemic and hyperuricemic-allopurinol treated groups.

	Control	**Hyperuricemic**	**Hyperuricemic allopurinol-treated**
UA (mg\dl)	1.4 ± 0.04	3.63 ± 0.16 *	1.51 ± 0.12#
Urea (mg\dl)	36.1 ± 9.7	37.7 ± 1.58	37.3 ± 1.89
Creatinine (mg\dl)	0.63 ± 0.05	0.79 ± 0.05	0.62 ± 0.05
Plasma MDA (nmol /ml)	5.33 ± 0.44	20 ± 1.7*	11.6 ± 1.1#
Erythrocytes SOD activity (U/ml)	1.59 ± 0.2	0.27 ± 0.02*	0.55 ± 0.06

UA: Uric acid   MDA: malondialdehyde   SOD: superoxide dismutase
* Significantly changed (p value < 0.05) when compared to the corresponding value in control group.
# significantly changed (p value < 0.05) when compared to the corresponding value in Hyperuricemic group.

Blood pressure changes:

Figure 1 shows representative examples of systolic blood pressure in all study groups. The figure shows that the systolic blood pressure of hyperuricemic group (102 ± 2.91 mmHg) was significantly (P<0.01) higher than the corresponding values in control group (70 ± 2.58 mmHg). The systolic blood pressure in hyperuricemic-allopurinol treated group (78 ± 1.83 mmHg) was significantly reduced (P<0.01) when compared to the corresponding value of hyperuricemic group.

Spontaneous uterine contractions:

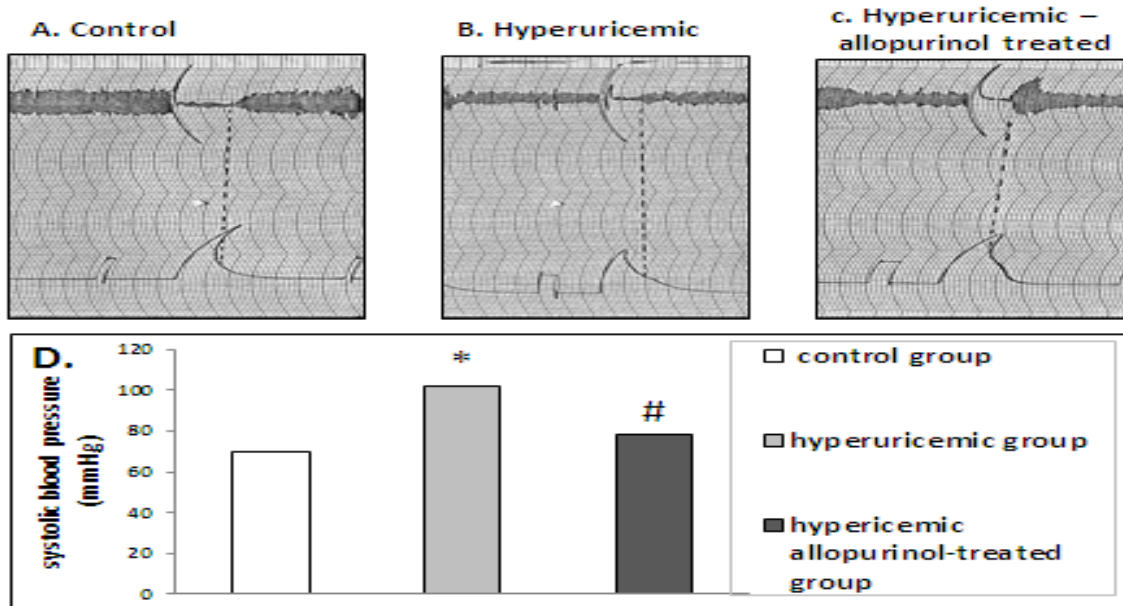Figure 2 shows representative examples of spontaneous uterine contractions in all study groups. The figure shows that in hyperuricemic group the frequency and the amplitude of spontaneous uterine contractions (6.2 ± 0.44 cycle\5 min. & 2.01 ± 0.3 cm respectively) were insignificantly changed (P>0.05) when compared to the corresponding values (6.8 ± 0.41 cycle\5 min. & 1.66 ± 0.24 cm) in control group. Also in hyperuricemic-allopurinol treated group the frequency and the amplitude of spontaneous uterine contractions (7.5 ± 0.07 cycle\5 min. & 2.4 ± 0.43 cm respectively) were insignificantly changed (P>0.05) when compared to the corresponding values in hyperuricemic group.

Figure (1): Representative examples of recording of the systolic blood pressure (mmHg) in A- Control group. B- Hyperuricemic group. C. Hyperuricemic allopurinol-treated group. D. Histogram showing systolic blood pressure (mmHg) in control, hyperuricemic and hyperuricemic-allopurinol treated groups.
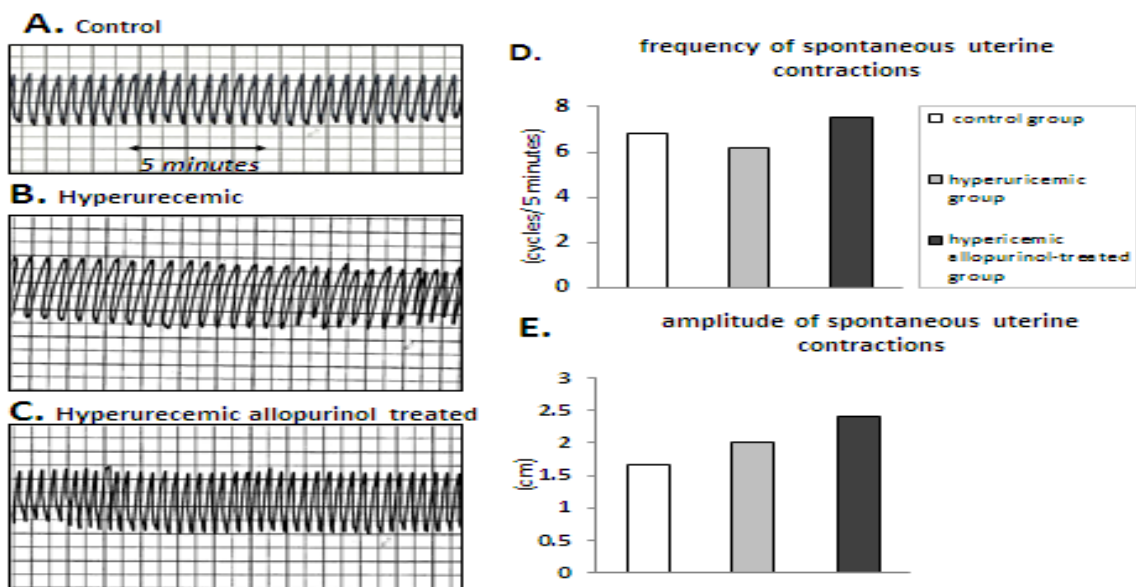
Figure (2): Representative examples of spontaneous uterine contractions in A- Control group. B- Hyperuricemic group. C. Hyperuricemic allopurinol-treated group. D. Histogram showing frequency of spontaneous uterine contraction (cycles /5 minutes) in control, hyperuricemic and hyperuricemic-allopurinol treated groups. E. Histogram showing amplitude of spontaneous uterine contraction (cm) in control, hyperuricemic and hyperuricemic-allopurinol treated groups.
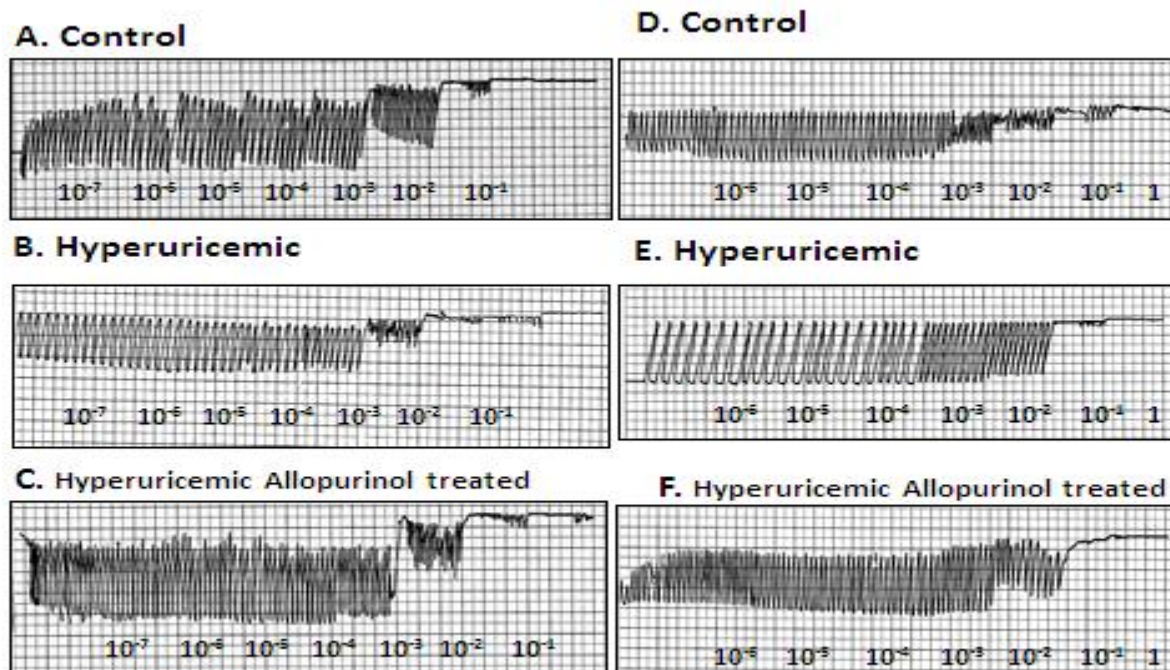

Figure (3): Representative examples of the uterine reactivity to cumulative doses of Ach from 10^{-7} to 10^{-1} M added to an organ path 40 ml capacity in A. control B. hyperuricemic C. hyperuricemic-allopurinol treated groups. And uterine reactivity to cumulative doses of oxytocin from 10^{-6} to 1 IU/ml added to an organ path 40 ml capacity in D. control E. hyperuricemic F. hyperuricemic-allopurinol treated groups.

Uterine reactivity to cumulative doses of Ach and oxytocin in hyperuricemia:

On the other hand Figure 3 shows representative examples of the uterine reactivity to cumulative doses of Ach and uterine reactivity to cumulative doses of oxytocin. The results show insignificant changes (P>0.05) in these parameters among the three groups.

DISCUSSION

Hyperuricemia has been considered as an important risk factor for gout and may be associated with oxidative stress conditions such as cardiovascular diseases [11]. Also, it was associated with increased risk of prematurity. The risk of adverse maternal and fetal outcome increased with increasing concentration of UA in hypertensive pregnancy [14].

The study showed that the induced hyperuricemia produced oxidative stress as indicated by significant increase in the level of plasma MDA and significant decrease in SOD activity. Also, systolic blood pressure was found to be significantly increased. Allopurinol treated groups show reversal of these parameters with reduction of oxidants and blood pressure. However renal function which was assessed by serum urea and creatinine showed insignificant change among groups, also uterine spontaneous contractions and uterine reactivity to cumulative doses of Ach and uterine reactivity to cumulative doses of oxytocin did not show significant changes. Rats were confirmed to be hyperuricemic by the elevation of serum uric acid, which was reduced by allopurinol treatment.

Oxidative stress can be defined as an imbalance between the oxidant and antioxidant system, with deviation towards the oxidant system. Hyperuricemia is suggested to be linked to oxidative stress because their levels were found to be elevated in this study. Increased UA level has been associated with increased production of oxygen free radicals; due to the conversion of xanthine dehydrogenase (XDH) to xanthine oxidase (XO) that plays a pivotal role in progression of oxidative stress condition [20]. XO is a source of ROS and may explain the link between hyperuricemia and oxidative stress-induced diseases [11, 20]. Moreover, the detected serum UA elevation may promote oxygenation of LDL-C and facilitate lipid peroxidation [4] and this may explain the elevation of MDA in this study as MDA is resultant from lipid peroxidation. Previous studies have concluded several mechanisms for the increase of oxidative stress with hyperuricemia. *Dalbeth and So,* postulated that Monosodium urate crystals initially trigger formation of the 'inflammasome' by its effect on cells of the monocyte/macrophage lineage. The detailed mechanism is not known, but cell damage leading to ATP release and activation of the P2X7 receptor which is implicated in ATP-mediated inflammation, may be involved [21]. Associated Potassium efflux may also be important, as well as generation of ROS. Also *George and Struthers*, found that the enzymes involved in UA production are also responsible for oxidative stress [22].

The elevated urate, MDA as an oxidative marker and the reduction in SOD in our study, explain the elevation of blood pressure in the hyperurecemic rats. Animal models have shown that acute elevations of serum urate (e.g., by inhibition of uricase) induce a prompt rise in blood pressure and that chronic urate elevation maintains the rise in

pressure and induces irreversible vascular damage and glomerular changes, and results in a form of salt-sensitive hypertension [23]. The mechanisms suggested are rennin angiotensin aldosterone dependent arteriolopathy, inhibition of neuronal nitric oxide synthase, and interstitial fibrosis and glomerulosclerosis with albuminuria [24].

Moreover, hyperuricemia promotes free radical formation, which stimulates the lipid peroxidation that increases the thickness of intima [25]. Furthermore, oxidized LDL has been implicated in the inhibition of endothelial NO synthase transcription and expression [26]. The elevated MDA level in this study affects vascular function which was agreed by *Golbidi and Laher,* who found that ROS-induced lipid peroxidation alters the structure and the fluidity of biological membranes, which ultimately affect vascular function [27]. UA via organic transporters enters the vascular smooth muscle cells and activates mitogen activated protein kinase and nuclear transcription factors, which result in proliferation of vascular smooth muscle cells and consequent upregulation of inflammatory mediators [28, 29]. The high serum UA level promotes platelet aggregation in vessel, which initiates cardiovascular disorders [30]. The concentrations of triglycerides, apolipoprotein B and apolipoprotein E are increased and the level of high density lipoproteins gets decreased in hyperuricemia that ultimately lead to atherosclerosis [31].

Blocking XO-generated oxygen radical accumulation has emerged as an intriguing new treatment option for preventing oxygen radical accumulation and its adverse effects [32]. Allopurinol which lower urate via inhibition of

XO, leads to decreased production of ROS, which may have contributed to any apparent beneficial effect [24]. However, on the other hand, insignificant increase in the mean value of SOD activity by concurrent administration of allopurinol coincides with Haidari et al, 2009 and Haidari et al, 2011 [15, 33] who reported that, allopurinol treatment could not significantly increase serum total antioxidant capacity.

Oxonic acid induced hyperuricemia with preservation of renal function agreed with *Mazzali and colleagues* who reported that administration of low doses of oxonic acid induced mild hyperuricemia (an increase of 1.5 to 2 fold in serum UA levels) without intrarenal urate crystal deposition that lead to acute renal failure [34]. This was approved by *Roncal et al.* who stated that mild hyperuricemia did not affect some renal function parameters (assessed by serum blood urea nitrogen and creatinine levels) or cause proteinuria [35].

In this study there were no direct effect between hyperuricemia and uterine contractions. Although other studies showed that serum uric acid is a marker for oxidative stress in preeclampsia which can result in diminished uterine contractility and impaired vascular relaxation also elevated serum uric acid in parturients undergoing cesarean delivery with neuraxial anesthesia correlated with increased use of supplemental uterotonic agents and decreased use of post-spinal vasopressors to minimize uterine atony and postpartum hemorrhage [36] which may point to a sort of relation between hyperuricemia and uterine contraction. This may be explained by the difference in response and contractions between the gravid and non-gravid uterus as previously

proved by *Ingram et al.,* who found that local influences regulate differential effects between the gravid and nongravid uterus of the pregnant tammar [37]. In eutherian mammals, changes in myometrial contractility during pregnancy and at the time of parturition are affected by the regulation of multiple pathways in uterine smooth muscle. The electrophysiology of muscle cells is dramatically altered during pregnancy, to limit excitability and elements of the signal transduction pathways for stimulatory factors are down-regulated, whereas those for inhibitory pathways are emphasized. Also, atrial natriuretic peptide (ANP) inhibited the tension development by myometrial tissues from oestrogen-treated virgin rats and the sterile horn of 10 to 14 day pregnant rats but not of the uterus from pregnant and progesterone-treated rats. Inhibition of cyclo-oxygenase and lipoxygenase activities did not restore the tocolytic activity of ANP on gravid uterus. ANP exerted a tocolytic effect on nongravid uterus submaximally stimulated by prostaglandin F2 alpha (PGF2 alpha), oxytocin, vasopressin, angiotensin II or 5-hydroxytryptamine (5-HT) [38].

The premature contraction and preterm labor associated with hyperuricemia may be caused by vascular lesions of the placenta which are commonly associated with these conditions, also stimulating the production of proinflammatory cytokines such as IL-1 and tumor necrosis factor alpha (TNF-α) by human decidua; these cytokines, in turn, stimulate the production of prostaglandins by the amnion and the decidua; the administration of IL-1 to pregnant mice or nonhuman primates induces preterm labor, which can be prevented by the administration of IL-1 receptor antagonist

protein. Similarly, proinflammatory cytokines up-regulate prostaglandin H synthase (PGHS) expression and down-regulate prostaglandin dehydrogenase (PGDH) expression leading to prostaglandin synthesis associated with preterm delivery [39].

So, further studies are recommended to find if hyperuricemia has different effect on gravid uterus or placental vasculature.

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