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Original Article

Effects of swimming exercise and RLN2 induction on relaxin levels, MMP-13, TGFβ expression in the pubic cartilage of pregnant rats

[Efectos del ejercicio de natación y la inducción de RLN2 sobre los niveles de relaxina, MMP-13, expresión de TGFβ en el cartílago púbico de ratas preñadas]

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Abstract

Context: Swimming exercises can be used as an intervention to maintain the stability of the synthesis of the pubic symphysis cartilage.

Aims: To evaluate the effects of swimming exercise on relaxin levels, MMP-13, TGFβ expression in pubic cartilage at pregnant rats with induction of RLN2 gene.

Methods: Thirty-six female Wistar rats (six in each group) were impregnated with 24 male rats (1:1). Three groups had swimming exercises for 60 min per day, and the other groups were treated as the control group. Two groups were given subcutaneous RLN2 gene. Relaxin plasma was collected from the sinus retro-orbital and performed twice on the eighth day and the 20th of pregnancy. The pubic symphysis of the rats was collected on the 20th day of pregnancy, and the decalcification process was carried out before making histological preparations.

Results: There was an increase in the amount of relaxin in the relaxin-induced group after 24 h induction (p=0.000). Relaxin levels of pregnant rats on the 20th day correlated with the expression of MMP-13, p=0.029; R^2=0.447. In the treatment group, relaxin levels and MMP-13 expression were lower than the non-treatment group, p=0.005 and p=0.000, respectively. In contrast, there was a higher expression of TGFβ in the treatment group, p=0.000.

Conclusions: Relaxin plasma levels have a significant correlation with the expression of MMP-13, and swimming exercise increases the expression of TGFβ and decreases relaxin levels and MMP-13 expression in the pubic cartilage.

Keywords: MMP-13; pregnant rat; pubic cartilage; relaxin; swimming exercise; TGFβ.

Resumen

Contexto: Los ejercicios de natación pueden ser utilizados como intervención para mantener la estabilidad de la síntesis del cartílago de la sínfisis púbica.

Objetivos: Evaluar los efectos del ejercicio de natación sobre los niveles de relaxina, MMP-13, expresión de TGFβ en cartílago púbico en ratas preñadas con inducción del gen RLN2.

Métodos: Treinta y seis ratas Wistar hembras (seis en cada grupo) fueron aparejadas con 24 ratas macho (1:1). Tres grupos realizaron ejercicios de natación durante 60 min por día, y los otros grupos fueron tratados como grupo de control. A dos grupos se les administró el gen RLN2 por vía subcutánea. El plasma de relaxina se recogió, del seno retroorbitario, el octavo día y el vigésimo día del embarazo. La sínfisis púbica de las ratas se recolectó el día 20 de gestación y se realizó el proceso de descalcificación antes de realizar las preparaciones histológicas.

Resultados: Hubo un aumento en la cantidad de relaxina en el grupo inducido por relaxina después de 24 h de inducción (p=0,000). Los niveles de relaxina de ratas preñadas en el día 20 se correlacionaron con la expresión de MMP-13, p=0,029; R^2=0,447. En el grupo de tratamiento, los niveles de relaxina y la expresión de MMP-13 fueron más bajos que en el grupo sin tratamiento, p=0,005 y p=0,000, respectivamente. Por el contrario, hubo una mayor expresión de TGFβ en el grupo de tratamiento, p=0,000.

Conclusiones: Los niveles plasmáticos de relaxina tienen una correlación significativa con la expresión de MMP-13, y el ejercicio de natación aumenta la expresión de TGFβ y disminuye los niveles de relaxina y la expresión de MMP-13 en el cartílago púbico.

Palabras Clave: cartílago púbico; ejercicio de natación; MMP-13; rata preñada; relaxina; TGFβ.

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INTRODUCTION

Pain in the pubic symphysis is often referred to as symphysis pubis dysfunction (SPD). It is experienced by 12% of patients in the first trimester, 34% in the second trimester, and 52% in the third trimester of pregnancy (Leadbetter et al., 2006). Pregnant women complain of stinging pain around pubic symphysis and the sacroiliac joint that often soreness down the thigh. This pain increases with daily activities such as climbing stairs, walking, standing, carrying heavy objects, changing sleep positions until unable to walk (Herren et al., 2015).

The pain level is related to the degree of widening of the interpubic disc. The widening of the pubic symphysis exceeding 6.3 mm begins to show mild symptoms, and if it expands more than 10 mm, it leads to pathology (Segal and Chu, 2015). The changes in the pubic symphysis during pregnancy in human is similar in mice, as confirmed by the results of histological and morphometric studies of the pubic symphysis in pregnant mice that also experience structural changes that widened pubic symphysis between 0.2 mm to 3 mm at the end of pregnancy (Pinheiro et al., 2004).

Relaxin produced by the corpus luteum and placenta has an essential role in dilating the interpubic disc (Wang et al., 2021) and collagen catabolism in the pubic symphysis of mammals. High relaxin levels promote reduced collagen content through collagen degradation by activating the collagenolytic system (Samuel et al., 1998). This mechanism is known based on studies that have been carried out on fibrocartilage tissues such as the anterior cruciate ligament, and it showed relaxin binds to specific receptors, further inducing the collagenases pathway that decreases alpha-smooth muscle actin (αSMA) and reduces transforming growth factor-beta (TGFβ) activity by inhibiting the pSmad2 pathway (Dragoo et al., 2009; Konopka et al., 2016). The intracellular collagenolytic pathway most closely associated with cartilage collagen is collagenase-3, which increases matrix metalloproteinase-13. MMP-13 degrades collagen II as the main structure of cartilage collagen (Kapila et al., 2009). The correlation between relaxin plasma levels and MMP-13 expression of pubic symphysis cartilage in pregnant rats has not been studied.

Collagen structures provide stability in the pubic symphysis and neutralize shear and tensile stresses caused by movements of normal daily activities (Becker et al., 2010). As the pregnancy goes, the fetus's weight also increases the shear load and tensile load on the symphysis, followed by an increased relaxin production that increases joint mobility, which causes mild to severe symptoms.

There is no based-on-scientific papers standard on how to deal with pregnancy-associated diastasis pubic pain. In severe cases, the patients will be injected by intra-symphyseal injection with hydrocortisone, chymotrypsin, and lidocaine once a day for 3 to 7 days (Cassagrande et al., 2015). Unfortunately, this symptomatic drug administration has often been avoided during pregnancy to prevent its impact on the fetus. Therapies have begun to be directed at the non-invasive procedure to eliminate the harmful effect for the fetus, such as pregnant fitness Pilates exercises (Oktaviani, 2018), Kegels exercises, gluteus maximus activation, and pelvic belt (Howell, 2012). Some of these studies that light exercise can reduce soreness; however, some have not focused on changes in the extracellular matrix/ECM and chondrocytes in pubic symphysis cartilage.

Studies on exercise therapy in pregnancy aimed at improving the balance of cartilage synthesis and degradation have not been investigated by researchers. Therefore, swimming exercises may be more appropriate to use in a laboratory study towards experimental rats to prove the changes in the symphysis pubic cartilage. Swimming exercises have the advantage of buoyancy and hydrostatic pressure (Baines and Murphy, 2010), which causes less muscle activity, less joint fatigue, and reduces mechanical stress on the pubic symphysis. Swimming is considered a light activity that can improve intra-cartilage structure through increased TGFβ and inhibit activation of MMP-13. Thus, this study aimed to investigate the effects of swimming exercises in the pubic symphysis cartilage on pregnant rat models. We also aim to determine the correlation of RLN2/prorelaxin induction on the relaxin plasma levels and its correlation with MMP-13 expression on the pubic symphysis cartilage. The results of this study might provide suggestions to clinicians and health practitioners to opt for swimming exercises as one of the management for pregnant women with SPD.

MATERIAL AND METHODS

Chemicals and reagents

Reagent and kits used were the rat relaxin ELISA Kit (CSB-EL019749Ra) from Cusabio, Houston, USA), MMP-13 (72B-01) mouse monoclonal antibodies (Santa Cruz, sc 80200, California), TGFβ monoclonal antibodies (Abcam, ab27969, Cambridge). Prorelaxin H2 protein (RLN2 gene/prorelaxin H2 protein) was purchased from GeneScript USA Inc, Piscataway

NJ08854, USA, catalog number: SC 1208; Chemical peptide synthesis: peptide 2: quantity 4 mg; purify >70%; length 29; sequence B chain: DSWMEEVIKLC GRELVRAQIAICGMSTWS and peptide 3: quantity 4 mg; purify >70%; length 24; sequence A chain: QLYSALANKCCHVGCTKRSLARFC, (GeneScript, protein sequence retrieved from Uniprot, available online at https://www.uniprot.org/uniprot/P04090).

Study design and animals

This research was an experimental study with a post-test only and control group design (Fig. 1). Experimental animals were 36 female rats Wistar strain, age 9-10 weeks, weight 110-150 grams. The female rats were required to meet the inclusion criteria: nulliparous female rats with healthy limb movement. The exclusion criteria were infection, abortion history, and death. The female was impregnated with 24 male rats aged 10 to 12 weeks. Rats were bred in the animal laboratory of the Faculty of Medicine, Universitas Brawijaya Malang, East Java, Indonesia. Thirty-six female rats were placed in each 40 × 50 × 20 cm cage, with room temperature between 22 ± 2.2°C, dark: light cycle 12:12 h (start from 06.30 am). The bedding and drinking tubes were renewed and restocked every day. The fed was restocked every afternoon at 04.00 pm. The regulation of light cycle and noise was following the ethical standard of experimental animal care. The experimental procedures that included animals have been approved by the Animal Research Ethics Committee of Universitas Brawijaya (Ethical Clearance No. 1165-KEP-UB).

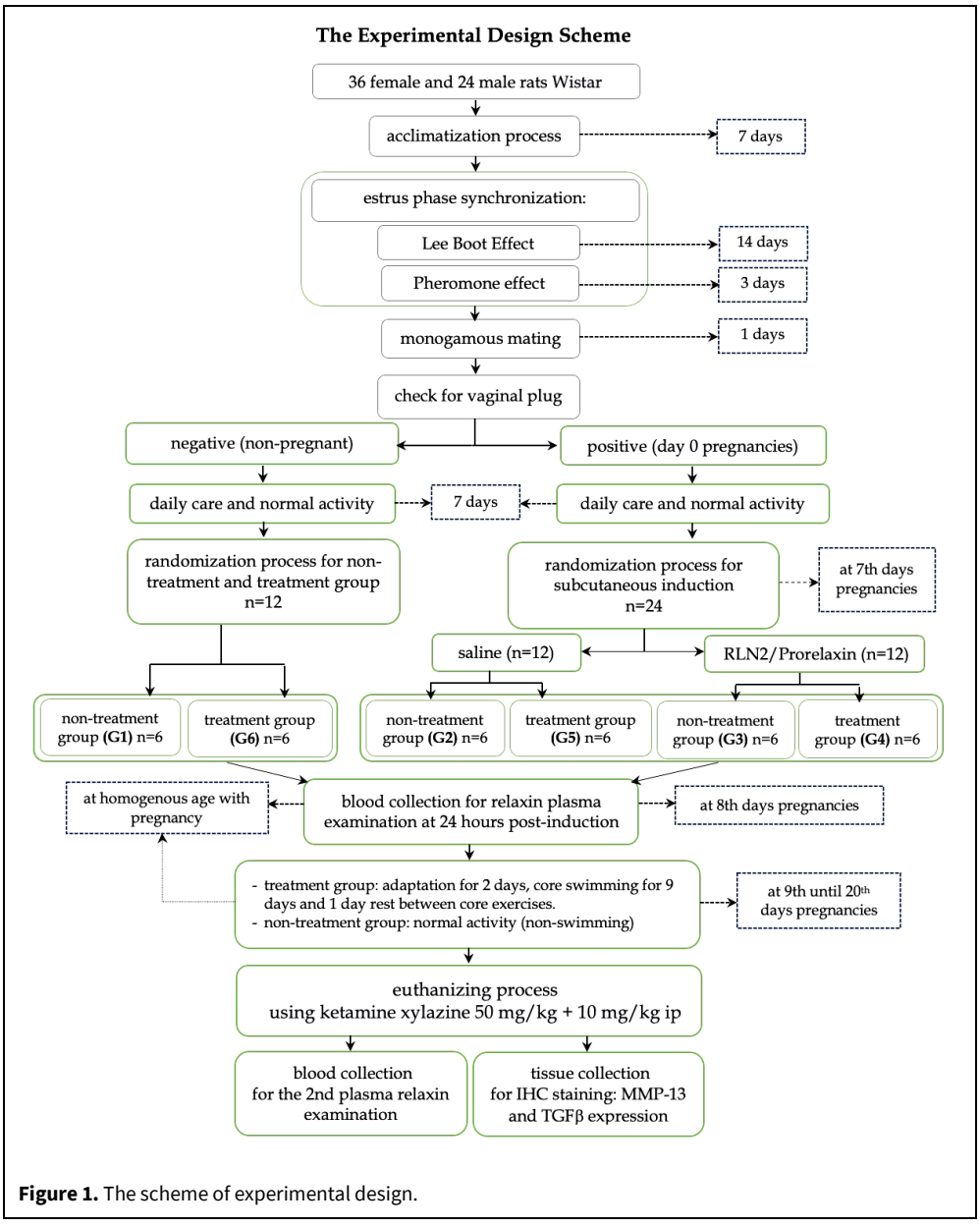

Figure 1. The scheme of experimental design.

Mating procedure and impregnation

Rats were acclimatized for seven days to reduce stress probability. Then, the estrus cycle of rats was synchronized using Lee Boot Effect and Pheromone Effect (Sardjono et al., 2019). Female rats, which were later confirmed to be in late proestrus and early estrus, were placed to mate with male rats overnight (monogamous mating). Day 0 pregnancies determined if there is rat sperm in female rats vaginal plug (Netto et al., 2020). The pregnancy was also confirmed by abdominal palpation on the 7th day after the mating.

Experimental group and induction of saline and relaxin

The G1, G2, G3 were control groups. The experimental groups were G4, G5, and G6. The criteria of each group were: G1 (negative control, nonpregnant rats); G2 (1st positive control group, pregnant rats with saline induction); G3 (2nd positive control group/non-treatment group, pregnant rats with relaxin induction); G4 (treatment group, pregnant rats, with relaxin induction); G5 (treatment group, pregnant rats, with saline induction); G6 (treatment group, nonpregnant rats).

The induction of saline and relaxin was carried out on the 7th day of pregnancy. Relaxin antigen was induced subcutaneously to groups G3 and G4 at a dose of 0.268 mg/kg. This dose has been confirmed to trigger the widening of the pubic symphysis after 24 h of induction (Zimmerman et al., 2017). The physiological fluid induction/saline induction was induced to the rats subcutaneously at a dose of 0.1 mL for the groups that received saline induction group (G2 and G5).

The relaxin induction is a safe compound for fetuses, even promoting adequate uteroplacental perfusion to allow for normal fetal and placental growth. Several previous studies reported that exogenous relaxin administration decreased uterine artery stiffness, increased blood flow velocity, and mediated uterine artery remodeling in pregnancy rats (Gooi et al., 2013; Vodstrcil et al., 2012).

Swimming exercise

Swimming treatment was applied to 3 different groups with uniform intensity: G4, G5, and G6. Swimming exercises were carried out on the 9th day of the pregnant rats and homogeneous age for the nonpregnant group. The swimming exercise was performed regularly once per day. For adaptation, the exercise started with light swimming activities for two consecutive days, for 10 and 15 min, respectively. Later on, the exercise duration was prolonged with a core swimming exercise of 60 min (Shen et al., 2013).

The core swimming exercise was separated into two steps: five days step one and four days for step two. Each step was separated by one gap day without swimming exercise. The core exercise for the first two days (in step one) was adjusted to the rat's ability to swim. If the rats could not float on the water surface or the rat showed the behavior of drowning its mouth 3-4 times during exercise, the treatment was stopped. The rats were then replaced and dried using a towel and hairdryer. It is essential to pay attention to the swimming ability of rats to maintain the quality of exercise in each group. Rats should have more than 60% ability to actively move the front and back extremities during swimming exercises (Kregel et al., 2006).

The size of the water tank used in this study was 74 cm (length) × 53 cm (width) × 60 cm (height). It had a 43 cm depth (Chen et al., 2012) and a water temperature of 34 ± 0.5°C. The water depth must exceed the rat's body length plus its tail to avoid the tail touching the base of the water tank. Importantly, rats were dried immediately and placed in a warm environment. This type of this pool was different from the one used by some researchers for force swim tests (FST), which is frequently used in models to induce and observe depression behavior (Slattery and Cryan, 2012; Yahav et al. 2015). The size of the pool was also can stimulate the rats to behave actively swimming and making horizontal swim movements throughout the water tank, which also includes crossing into another quadrant.

One sign of rats having stress or inability to defend themselves is shown by passive or immobility behavior such as floating that is longer than active swimming (Slattery and Cryan, 2012). Researchers confirmed no signs of floating/immobility behavior that exceeded 10% of the whole core workout (Kregel et al., 2006). Tired animals that appeared distressed were allowed to rest for 5 min before finishing the swim session (Shen et al., 2013). Setting the type and size of the water tank, water temperature, and stages of adaptation before the core swim were any efforts by researchers to prevent stress in experimental animals (Matinfar et al., 2021). This training protocol and the water temperature were designed to mimic clinically relevant physical therapy programs (Shen et al., 2013).

Blood collection for relaxin plasma examination

The blood was collected from the sinus retroorbital and performed was carried out twice, on the 8th day and on the 20th of pregnancy of each rat (the nonpregnant rats were treated the same as well). Venous blood samples were drawn into ethylenediaminetetraacetic acid-plasma tubes and arrived at the

laboratory the same day. Plasma was separated from blood cells by centrifugation at 2000 rpm for 10 min and was stored at −70°C until use. Relaxin plasma levels was examined using the ELISA kit rat relaxin (Cusabio, CSB-EL019749Ra).

Tissue collection

The rat's tissues were collected for further experiment. On the 20th day of pregnancy, pregnant rats that belonged to treatment groups were dissected. The dissection was performed for a minimum of 6 h after swimming exercise on the last day. For the group without swimming or control groups (G1-G3), dissection was also performed at the same time.

The rats were euthanized using ketamine xylazine 50 mg/kg + 10 mg/kg ip. The pelvic and pubic symphysis tissue was collected, and then the soft tissues were carefully separated around the observation tissue to avoid disturbance of the ligaments and interpubic disc. The tissue was then fixed in 10% formalin solution for a maximum of 3 days and stored at room temperature before the preparation of the histological observation.

The process of tissue preparation for histological observation and immunohistochemical process

Before tissue preparation (dehydration, clearing, paraffin infiltration, and embedding), the materials were processed through decalcification using a Rapid Cal Immuno fluid, where the sample was immersed in a Rapid Cal Immuno-solution with a tissue volume of 30 to 60% for three days. After the embedding process, the materials were sliced into thin microscope-observable layers of 2-5 microns. After the observation slides had been ready for immunohistochemical stained, the samples were then stained using MMP-13 (72B-01) mouse monoclonal antibodies (Santacruz, sc 80200), TGFβ monoclonal antibodies (Abcam, ab27969).

Each sample was assessed semi-quantitatively according to the immunoreaction score (IRS). The score was obtained from 5× microscope observation (replication on the different observation views) using Nikon e100 with a 12.5 MP OptiLab digital camera (magnification = 400×) and 3.0 Raster Image processing software.

Statistical analysis

Data were analyzed based on the Kruskal Wallis test and One-way analysis of variance (ANOVA), then further analyzed with *post-hoc* Bonferroni's, Dunn's, and Tukey test using Graphpad Prism 9.0 (version 9.0) and IBM SPSS software (version 25, USA). The final score was represented by mean value ± standard deviation (SD) of n = 6, with p significance at ≤0.05. Analysis of differences in plasma relaxin levels in the nonpregnant group, we analyzed using the Mann-Whitney test with p significant at ≤0.05. In addition, the strength of correlation between relaxin plasma levels and expression of MMP-13 on symphysis was measured with Spearman, the $p<0.05$ was considered statistically significant.

RESULTS

Relaxin plasma levels after RLN2 gene induction and swim exercise

Induction of (RLN2) prorelaxin H2 protein at a dose of 0.268 mg/kg subcutaneously was administered to G3 and G4 showed higher relaxin plasma levels than the saline-induced groups (G2 and G5). *Post hoc* analyses revealed a significant difference in relaxin plasma levels between the pregnant rats with relaxin and saline induction (Fig. 2A).

The mean relaxin in groups G3 and G4 at 24 h post-induction/ (d-8) were 843.53 ± 134.31 pg/mL and 846.12 ± 136.99 pg/mL (mean ± SD), while the mean of relaxin plasma levels in the pregnant group with saline induced (G2 and G5) were 325.25 ± 90.33 pg/mL and 333.83 ± 69.34 pg/mL (mean ± SD), respectively.

According to Fig. 2B, the results of measuring relaxin plasma levels on the 20th day in the treatment group (G5 and G4) showed lower than the non-treatment (G2 and G3). Relaxin in the G5 and G4 showed levels of 451.23 ± 98.53 pg/mL and 627.17 ± 25.85 pg/mL (mean ± SD), respectively. In contrast, The G2 and G3 showed higher levels, which were 610.61 ± 106.80 pg/mL and 775.98 ± 166.45 pg/mL, respectively. *Post hoc* analyses showed a significant difference between the treatment and non-treatment groups. Meanwhile, for the group of nonpregnant rats (Fig. 2C) based on the Mann-Whitney statistical test, there was no significant difference in plasma relaxin levels between groups G1 and G6 either before treatment (at eighth day) or after swimming treatment (at 20th day).

Correlation between relaxin plasma levels and MMP-13 expression

According to Fig. 3B, plasma relaxin levels at the 20th-day pregnancy group correlated with MMP-13 expression in the pubic symphysis tissue $p=0.029$, $R^2=0.447$. While relaxin levels on the eighth day of pregnant rats showed no correlation with MMP-13 expression in pubic symphysis cartilage, $p=0.710$ as stated in Fig. 3A. Meanwhile, in the group of non-pregnant rats, there was no correlation between relax-

in plasma levels and MMP-13 expression, both before treatment (d-8) and post-treatment (d-20) (Fig. 3C-D).

MMP-13 expression in pubic symphysis cartilage

The highest MMP13 expression value was the G3, and the lowest MMP-13 expression was the G1. The results showed that swimming exercise significantly reduced the expression levels of MMP-13 in the pubic symphysis cartilage. Kruskal Wallis statistical test showed a significant difference for all groups p=0.000, and *post hoc* analysis showed a different notation (Fig. 4).

Based on Fig. 5, MMP-13 expression was detected in the matrix in all groups but seemed a stronger and deeper expression in the matrix and chondrocytes in the G2 and G3. While the group with the swimming treatment (G4, G5, G6) gave a lower expression score than the control group.

TGFβ expression in pubic symphysis cartilage

According to Fig. 6, the highest score of TGFβ expression was the G4, and the lowest score was the G3. The treatment group showed mean score expression was higher than the positive controls (G2 and G3) and the negative control (G1). The statistical test showed significant differences for all groups p=0.000 (one-way ANOVA), and post hoc Tukey showed differences between the treatment and control groups, respectively. This study showed that swim exercise could increase TGFβ expression in pubic symphysis cartilage.

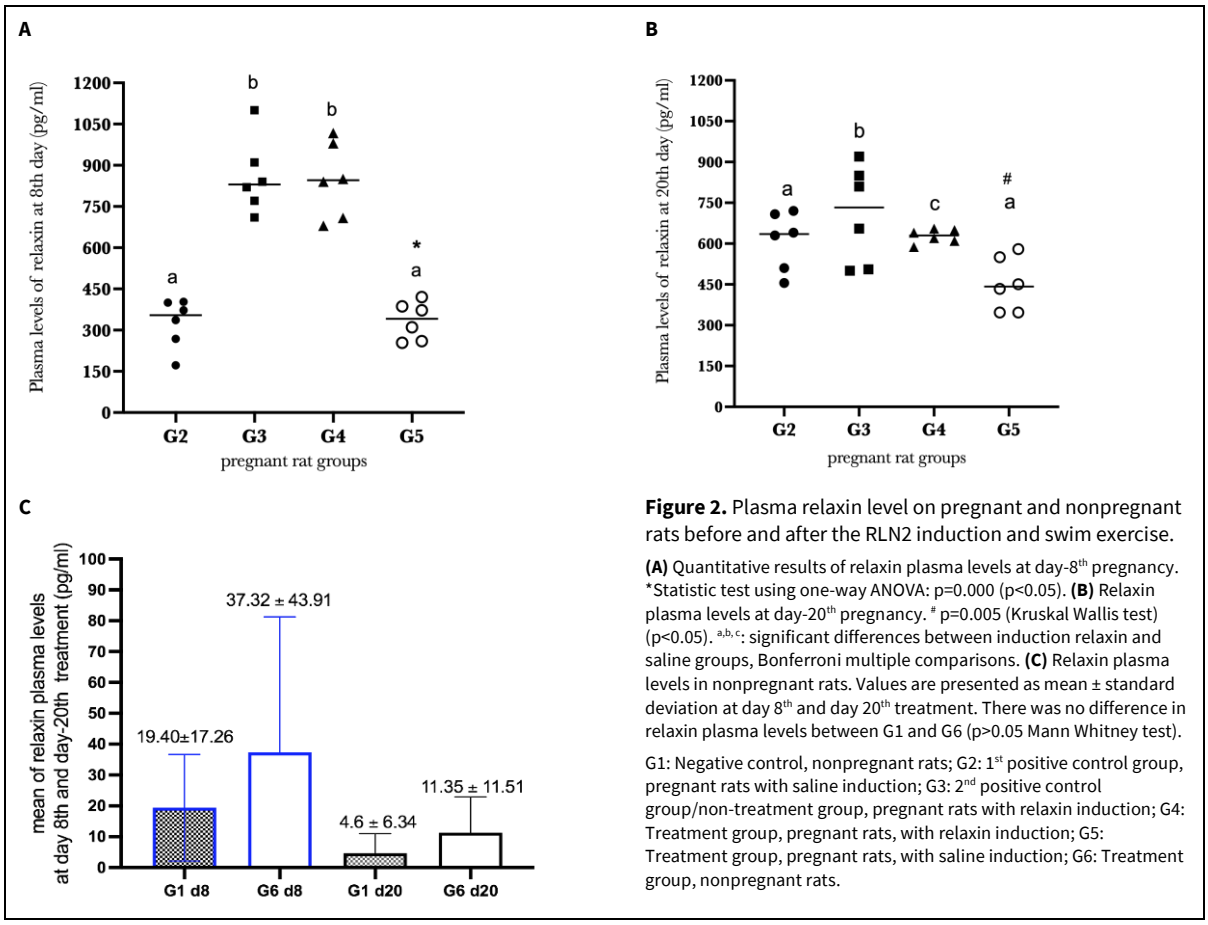

Figure 2. Plasma relaxin level on pregnant and nonpregnant rats before and after the RLN2 induction and swim exercise.

(A) Quantitative results of relaxin plasma levels at day-8th pregnancy. *Statistic test using one-way ANOVA: p=0.000 (p<0.05). **(B)** Relaxin plasma levels at day-20th pregnancy. # p=0.005 (Kruskal Wallis test) (p<0.05). a,b,c: significant differences between induction relaxin and saline groups, Bonferroni multiple comparisons. **(C)** Relaxin plasma levels in nonpregnant rats. Values are presented as mean ± standard deviation at day 8th and day 20th treatment. There was no difference in relaxin plasma levels between G1 and G6 (p>0.05 Mann Whitney test).

G1: Negative control, nonpregnant rats; G2: 1st positive control group, pregnant rats with saline induction; G3: 2nd positive control group/non-treatment group, pregnant rats with relaxin induction; G4: Treatment group, pregnant rats, with relaxin induction; G5: Treatment group, pregnant rats, with saline induction; G6: Treatment group, nonpregnant rats.

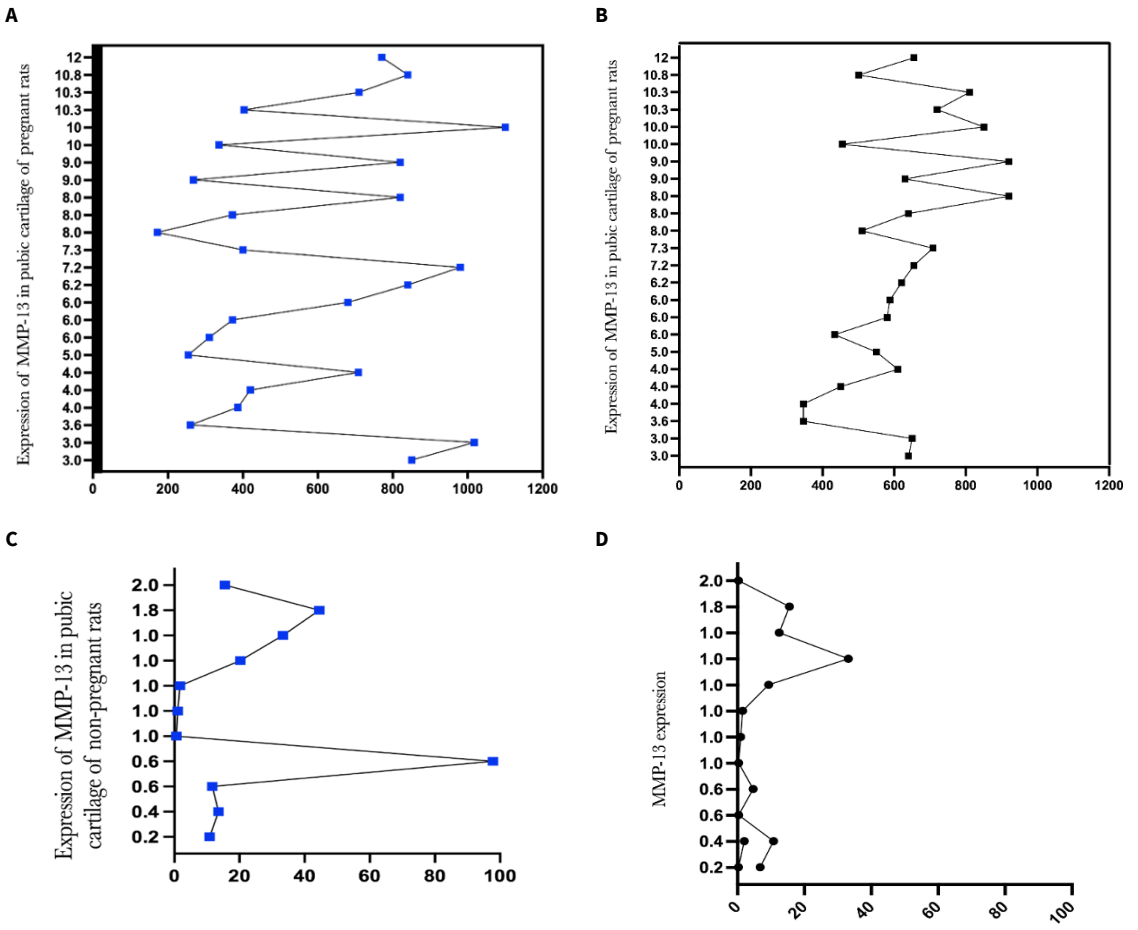

Figure 3. Correlation of relaxin plasma levels (pg/mL) measurements by ELISA kit and expression of MMP13 in pubic symphysis cartilage measurement by IHC staining.

(A) Correlation relaxin plasma levels at day 8th pregnancy and MMP-13 expression, there was no correlation, p>0.05 (Spearman correlation test). **(B)** Significant correlation between relaxin plasma level at day 20th pregnancy and MMP-13 expression (R^2 = 0.447). **(C)** and **(D)** Correlation of relaxin plasma levels and MMP-13 expression at day 8th and 20th treatment (no significant correlation, p >0.05).

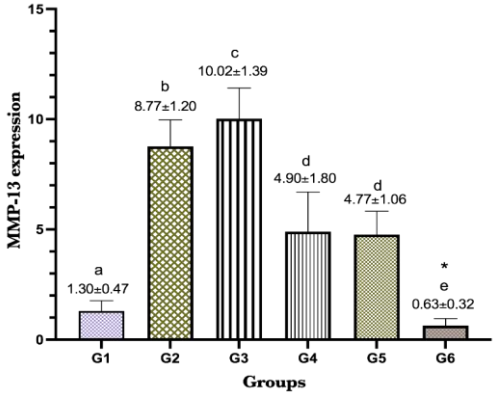

Figure 4. MMP-13 expression in pubic symphysis cartilage.

Values are expressed as mean ± SD (n = 6), *p<0.05 statistically significant differences (Kruskal Wallis). and *post hoc* Dunn's test indicated by different letters.

G1: Negative control, nonpregnant rats; G2: 1st positive control group, pregnant rats with saline induction; G3: 2nd positive control group/non-treatment group, pregnant rats with relaxin induction; G4: Treatment group, pregnant rats, with relaxin induction; G5: Treatment group, pregnant rats, with saline induction; G6: Treatment group, nonpregnant rats.

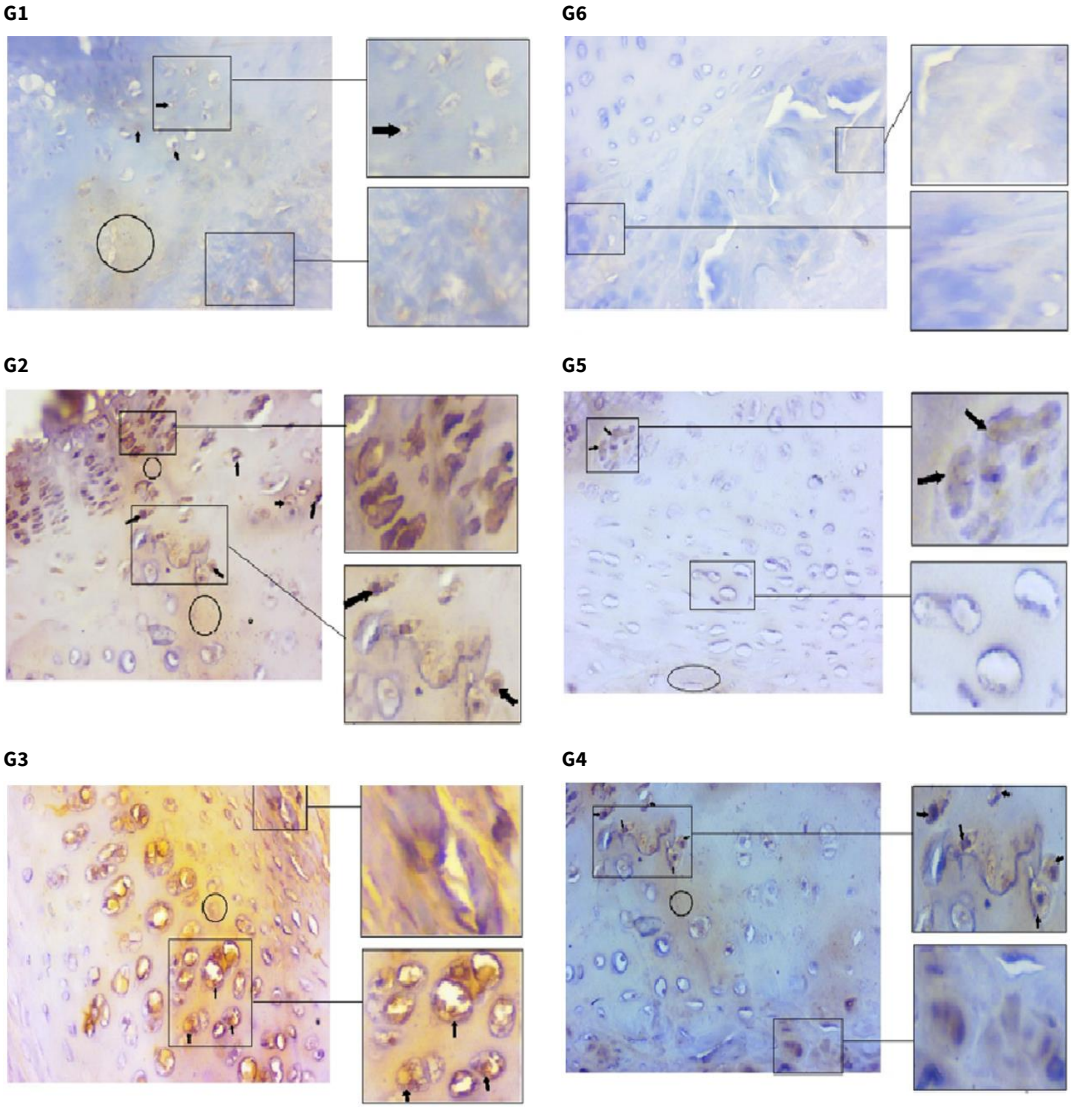

Figure 5. MMP-13 expression in symphysis pubic cartilage of all groups.

Immunohistochemical staining; 400× magnification; Nikon e100 with a 12.5 MP OptiLab digital camera; Olympus Olyvia viewer for imaging application and 3.0 Raster Image processing software. Black shape: positive expression in the chondrocyte; Circle: positive expression in the extracellular matrix.

G1: Negative control, nonpregnant rats; **G2**: 1st positive control group, pregnant rats with saline induction; **G3**: 2nd positive control group/non-treatment group, pregnant rats with relaxin induction; **G4**: Treatment group, pregnant rats, with relaxin induction; **G5**: Treatment group, pregnant rats, with saline induction; **G6**: Treatment group, nonpregnant rats.

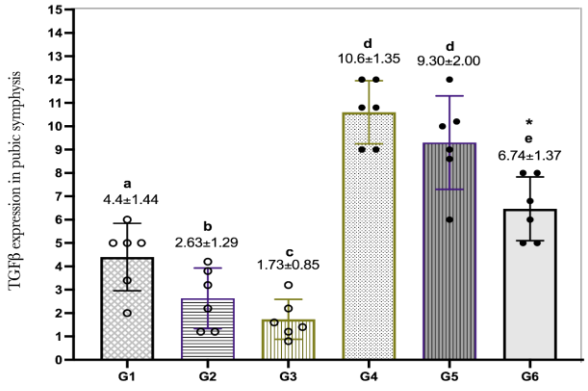

Figure 6. TGFβ expression in the pubic symphysis cartilage.

Values are expressed as mean ± SD (n=6), *p<0.05 statistically significant differences (one-way ANOVA), and *post hoc* Tukey test indicated by different notation.

G1: Negative control, nonpregnant rats; G2: 1st positive control group, pregnant rats with saline induction; G3: 2nd positive control group/non-treatment group, pregnant rats with relaxin induction; G4: Treatment group, pregnant rats, with relaxin induction; G5: Treatment group, pregnant rats, with saline induction; G6: Treatment group, nonpregnant rats.

DISCUSSION

The relaxin hormone physiologically increases in pregnancy involved in the widening process of the pubic symphysis to facilitate a safe delivery process in many species, including mice and humans (Pinheiro et al., 2004). The higher levels of relaxin in pregnant women provide a more substantial anti-fibrotic effect, which would cause ligament relaxation and damage, including interpubic (Wang et al., 2021). This study attempted to investigate the induction of RLN2 in pregnant rats, increasing relaxin plasma levels, and its correlation to MMP-13 expression in the pubic symphysis cartilage. In addition, it has been proven that swimming exercise keeps relaxin plasma levels from increasing excessively and improves the balance of synthesis and degradation of cartilage matrix by measuring the expression of TGFβ and MMP-13.

The dose of 0.268 mg/kg BW of gene RLN2 in this study significantly increased plasma relaxin levels higher than the group without relaxin induction. On the 20th day of pregnancy, relaxin plasma levels significantly correlated with MMP-13 expression in the pubic symphysis cartilage. This dose, adopted from the Zimmerman et al. (2017) study, changed the intrapubic ligament length in the mice to reach 0.5-2.5 mm (from microcomputer measurement).

Endogenous relaxin, which is an ovarian hormone, and exogenous RLN2 (prorelaxin H2 isoform 1 preproprotein), both of which would bind to relaxin receptors in the symphysis, were RXFP1 and RXFP2 (STITCH, 2021); these two receptors induced MMP-9 and MMP-13 and break down collagen in fibrochondrocytes of mice (Ahmad et al., 2012). This study showed a significant correlation between relaxin plasma levels and MMP-13 expression in symphysis tissue. It can be seen in the results that MMP-13 expression is more strongly expressed in conditions where relaxin levels were highest.

The fetus's weight also increases along with pregnancy, which causes the intensity and frequency of excessive pressure on the symphysis. This tension or mechanical stress will affect the matrix as a signal transducer to chondrocyte cells. Chondrocytes will synthesize and release IL-1 (Buckwalter, 2004) and TNF-α (Hogrefe et al., 2012) into the matrix. Chondrocytes can also bind to receptors on the cell surface through autocrine or paracrine activity and are trapped in the matrix. IL-1 stimulates chondrocytes to release destructive proteases (MMPs) involving MMP-13 as a component of type II collagen metalloprotease (Troberg and Nagase, 2012). TGFβ inhibits this activity; it is in charge of stimulating chondrocyte synthesis, matrix, cell proliferation and reducing the catabolic activity of IL-1 and TNF (Jiang et al., 2017).

In cartilage, TGFβ is stored in a latent form, which is synthesized as an inactive precursor, consisting of the mature ligand and latency-associated peptide (LAP). They are bound together until the adult ligand is released. Latent TGFβ binds to the extracellular matrix via latent TGFβ binding proteins (LTBPs). TGFβ activation is influenced by several mechanisms that cause interactions with the integrins cell surface. These mechanisms are physical, chemical, and enzymatic activities (Van Der Kraan, 2017).

Regular exercise will stimulate cartilage tissue, which quickly activates chondrocyte TGFβ signaling. That is because mechanical loading on the cartilage will either activate LAP or deactivate the bounding LAP-TGFβ. Furthermore, the mature TGFβ ligand will bind to the chondrocyte TGFβ receptor. However, the TGFβ without the receptor is rapidly no longer available in the extracellular matrix. The binding of active TGFβ to activin receptor-like kinase 5 (ALK5) stimulates the expression of latent TGFβ1 and ALK5 while downregulating the expression of ALK1. ALK5 stimulates SMAD2 and SMAD3 phosphorylation for blocking chondrocyte hypertrophy (Van Der Kraan, 2017).

In the case of trauma or cartilage suppression such as in SPD, it is suspected that there is an increase in IL-1β and TNFa levels in chondrocytes when the chondrocytes have high levels of IL-1β, it will increase SMAD7 expression, which is an inhibitor of intracellular TGFβ signaling via NF-κB activation. Swimming exercises have advantages of buoyancy, water viscosity and hydrostatic pressure, muscular endurance, effective pain control, and decreased muscle tension and injury (Baynes and Murphy 2010; Layne, 2015), which will reduce the compressive force on cartilage.

The hydrostatic pressure during exercise significantly affects the cardiovascular system and helps redistribute extracellular fluid back into the systemic circulation. Hydrostatic pressure has been shown to have a chondroprotective effect because it inhibits pro-inflammatory mediators in chondrocytes and regulates the synthesis of aggrecan and type II collagen (Carter et al., 2004). The result of this study can decrease MMP-13 expression in chondrocytes, which was similar to the study conducted by Milares et al. (2016). TGFβ has an important role in increasing tissue inhibitors of metalloproteinases/TIMPs. The role of TIMPs is to prevent excessive ECM degradation of MMPs (Kwak, 2013).

This result showed that swim exercise for 11 days in pregnant rats showed a significant difference in

plasma relaxin levels between the treatment and control groups. Nevertheless, it did not change in the nonpregnant group. The plasma concentration of many other peptides and hormones also changes during exercise (Richter, 1989), but a controversial theory within a study conducted by Schoenfeld et al. (2020) stated that physical exercise with hemodynamic changes did not cause significant changes in circulating relaxin.

It is necessary to conduct a longitudinal study about the dose and intensity of swim exercise on changes of relaxin hormonal and the expression of anti-inflammatory and pro-inflammatory cytokines in the pubic symphysis cartilage.

CONCLUSION

The results confirmed that the induction of the RLN2 gene was able to increase relaxin plasma levels in pregnant rats, and swimming exercise increased TGFβ and decreased MMP-13 expression in pubic symphysis tissue, and decreased relaxin plasma levels in pregnant rats. This activity allows to restore the balance of cartilage synthesis and degradation and reduce hypermobility of the symphysis joint during pregnancy.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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Contribution	Oktaviani I	Wihastuti TA	Rahardjo B	Wahyuni ES	Putra BA
Concepts or ideas	x	x	x	x	
Design	x	x	x	x	
Definition of intellectual content	x	x	x	x	x
Literature search	x	x	x	x	x
Experimental studies	x				
Data acquisition	x	x			x
Data analysis	x				x
Statistical analysis	x				
Manuscript preparation	x	x	x	x	x
Manuscript editing	x	x			
Manuscript review	x	x	x	x	x

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