Original Research

Estrous cycle phase affects myocardial infarction through reactive oxygen species and nitric oxide

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1. Abstract

Introduction: Myocardial infarction is the leading cause of death in women worldwide. Several studies have shown that estrogens play a cardioprotective role in women by decreasing reactive oxygen species (ROS) and increasing nitric oxide (NO). The aim of this work was to determine whether the evolution of myocardial infarction depends on the phase of the estrous cycle. **Methods**: Female Wistar rats were randomized into the following groups with an (n = 7 per group): (1) ovariectomized (OVX-sham); (2) OVX-48 h coronary occlusion (CO); (3) OVX-2 w CO;

Submitted: 14 April 2021 Revised: 27 August 2021 Accepted: 23 September 2021 Published: 30 December 2021

(4) proestrus-sham; (5) proestrus-48 h CO; (6) proestrus-2 w CO; (7) estrus-sham; (8) estrus-48 h CO; and (9) estrus-2 w CO. We measured the percentage of myocardial necrosis, cardiac hypertrophy, hemodynamic parameters, and the production of NO and ROS, after acute and chronic myocardial infarction was induced in proestrus or estrus or ovariectomized female rats. **Results**: The infarct area was reduced in the proestrus groups, while it was increased in the estrus and OVX groups. The left ventricular systolic pressure (LVSP) and $\pm$ dP/d*t* were reduced, but left ventricular diastolic pressure (LVDP) was increased in the OVX groups. NO was increased in the OVX + CO and estrus + CO groups. Production of ROS was increased in OVX rats after myocardial infarction but remained unchanged in proestrus and estrus. **Conclusion**: The phase of the estrous cycle in which the myocardial infarction occurs is important. When the coronary occlusion occurs during the proestrus phase, it prevents changes in cardiac function, the development of hypertrophy, oxidative stress and changes in NO levels, and reduces the extent of infarction.

2. Introduction

For many years, there has been a lack of investigations on cardiovascular diseases (CVD) in female subjects. Furthermore, gender differences have been neglected in the diagnosis and treatment of these conditions; consequently, there is an alarming increase in the incidence of CVD among women, including myocardial infarction (MI) [1, 2].

In general terms, women's mortality due to myocardial infarction has not been thoroughly considered or studied in depth. Globally, 521,900 deaths due to breast cancer were reported [3]; while 12.59 million deaths due to CVD occurred in the same year [4]. In addition, in clinical practice, there is a history of misdiagnosis and mistreatment of myocardial infarction in women [1]. This fact can be explained in part by the lower incidence of MI among premenopausal women compared to men of the same age, whereas the mortality rate in postmenopausal women is similar to or even exceeds that of men. This marked difference is attributed mostly to the role of sex hormones [5]. There is a body of evidence supporting that sex hormones play a cardioprotective role in young women.

Several studies have tested the cardioprotective effect of estrogens. However, few investigations [6] have associated the phases of the estrous cycle with MI. Most of the studies have simply focused on the presence or absence of hormones, but not on the physiological variation in premenopausal hormone levels. For instance, some clinical trials have shown that estrogen or estrogen-progesterone therapies in pre- and post-menopausal women have had no significant effect on preventing MI, while others demonstrated an association of low endogenous levels of estrogens and high levels of testosterone with cardiovascular risk and death [7–11].

17β-estradiol (E2) is well known for its protective effect on cardiovascular function, which is mediated by three receptors (ERs) [12], namely, ER-alpha (ERα), ER-beta (ERβ) and G protein-coupled estrogen receptor (GPER) [13, 14]. The presence of all of these receptors has been described in the heart, where acute administration of E2 prevents ischemic damage by modulating the production of nitric oxide (NO) and by decreasing the generation of reactive oxygen species (ROS) [15].

Three different isoforms of NO synthase (NOS) can generate NO. Neural NOS (nNOS) and endothelial NOS (eNOS) are expressed constitutively in neurons and endothelial cells, but can also be expressed in other cells and are related to cardiovascular homeostasis. These NOS are calcium-dependent and generate nanomolar concentrations of NO. Inducible NOS (iNOS), on the other hand, was discovered in macrophages and it has been related to inflammatory stages activated by cytokines; once activated, iNOS generates large quantities of NO [16]. Within this context, NO may have antiapoptotic, antihypertrophic, and cellular protective effects and may participate in cardiac muscle contraction [17, 18]. Also, it has been reported that an increase in stress factors such as ROS has been reported to influence the evolution of MI since these chemical species participate in cell damage signaling, necrosis and cellular apoptosis through their oxidizing effects on macromolecules such as lipids and proteins [19].

Therefore, this work aimed to determine whether the evolution of MI depends on the phase of the estrous cycle. To demonstrate this, we measured the percentage of myocardial necrosis, cardiac hypertrophy, hemodynamic parameters, and the production of NO and ROS after acute and chronic MI was induced during proestrus or estrus and compared the results to ovariectomized female rats.

3. Materials and methods

3.1 Animals

Female Wistar rats (12 to 13 weeks old) were obtained from the CINVESTAV-IPN Unidad Sur animal facility. All animal procedures were conducted according to Federal Regulation for Animal Experimentation and Care (SAGARPA, NOM-062-ZOO, 1999, Mexico), local ethics committee number C18_06 (CICUAE-FESC), and National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8023, revised 1978, USA). Animals were randomized into the following groups: (1) ovariectomized (ovx)-sham; (2) ovx-48 h coronary occlusion (CO); (3) ovx-2 w CO; (4) proestrus-sham; (5) proestrus-48 h CO; (6) proestrus-2 w CO; (7) estrus-sham; (8) estrus-48 h CO, and (9) estrus-2 w CO. In the ovariectomized groups, the animals were allowed to recover for 5 weeks after bilateral ovariectomy before CO. Forty-eight hours or 2 weeks after CO surgery, catheter-

ization was performed, plasma samples were obtained to quantify sex hormones, the left ventricular penumbra area was removed to measure NO by the Griess reaction, and ROS were quantified by electronic paramagnetic resonance (EPR).

3.2 Bilateral ovariectomy

Rats were anesthetized using pentobarbital (55 mg/kg i.p.) and subjected to bilateral ovariectomy as previously reported [20]. The same procedure, except for removal of the ovaries, was performed in the control (sham) group. All animals received postoperative analgesia with 0.1 mL of tramadol and topical erythromycin. At the end of the experiment, the uterus was removed, weighed and normalized to body weight for the evaluation of hormone depletion.

3.3 Vaginal smear

A vaginal smear was obtained to allocate the females to the estrus and proestrus groups in order to perform CO during the correct estrous phase (CO) [21]. A cannula with saline solution was gently and quickly inserted into the vaginal orifice; the introduction was shallowed (approximately 1 cm) to avoid cervical stimulation. Subsequently, a small amount of vaginal fluid was suctioned. Rats were not anesthetized during smear collection. The collected sample of epithelial cells was placed on a slide, dried at 37 °C, and fixed in an ethanol-ether solution (1:1) for one min, for subsequent staining with gentian violet. Thus, only rats in the estrus or proestrus cycle phase were occluded and allowed to develop MI while estrous cycle continued its course.

3.4 Coronary occlusion

Rats were anesthetized by i.p. administration of 40 mg/kg ketamine + 5 mg/kg xylazine. A thoracotomy was performed between the 4th and 5th intercostal space to exteriorize the heart, and breathing support was administered simultaneously. The left anterior coronary artery was located and ligated with an atraumatic needle and 5/0 silk thread. The heart was returned to the thoracic cavity. All animals were allowed to recover and received analgesics and topical erythromycin [22]. The animals were randomly allocated to the 48-h group or 2-week group.

3.5 Percentage of the infarcted area

At the end of each experiment, the animals were euthanized by cervical dislocation. The heart was cut into 2 mm sections and stained with 0.1% nitrotetrazolium blue chloride (NBT, Sigma Aldrich N6876) for 15 min to differentiate the non-infarcted (red tissue) from the infarcted area (white tissue). The infarcted area was calculated as the percentage of the total area of the left ventricle by densitometry using image processing software (Adobe® Photoshop CC 2019 20.0.10, Michigan, USA) and is expressed in arbitrary units. The evaluation was carried out in a blinded fashion.

3.6 Determination of cardiac hypertrophy

This parameter was calculated by the following formula: WIx = Wx/Wc, where WIx = wet weight index of the corresponding chamber; Wx = wet weight of the chamber, and Wc = animal body weight.

3.7 Cardiac catheterization

Forty-eight hours or 2 weeks after CO, the animals were anesthetized by i.p. administration of 35 mg/kg sodium pentobarbital. A tracheotomy was performed, and the right carotid artery was dissected and cannulated with a heparinized PE-10 catheter connected to a pressure transducer (Biopaq Systems, Santa Barbara California, USA). The catheter was inserted into the left ventricle to measure the left ventricular systolic pressure (LVSP), left ventricular diastolic pressure (LVDP), and the maximum range of isovolumetric pressure (+dP/d*t*max) and decay (–dP/d*t*max) as well as heart rate (HR) [23].

3.8 Quantification of 17β-estradiol and progesterone by ELISA

After the hemodynamic data experiments, blood samples were collected and serum was obtained to measure estradiol and progesterone levels using a commercial kit, following the manufacturer's specifications (Monobind Inc. ELISA Kit®, Lake Forest, California, USA).

3.9 Nitric oxide quantification

NO was quantified by the Griess method. Briefly, 100 mg of the left ventricle penumbra was used. The tissue was homogenized in 500 μL of 10% HCl and then centrifuged at 12,000 rpm for 5 min; next, 500 μL of the supernatant, 1.6 mL of N-ethylenediamine, 1.6 mL of 1% sulfanilamide, and 10% HCl were added to obtain a final volume of 5 mL. The samples were read in a UV-VIS spectrophotometer at λ = 540 nm (2800 UV/VIS Cole Parmer, Illinois, USA) [24].

3.10 Quantification of reactive oxygen species by electronic paramagnetic resonance (EPR)

Krebs/Hepes buffer (88.6%/1.4% w/v) containing 156.17 nM of 3.15% diethyldithiocarbamate acid silver salt (DETC) and 3.045 μM of 8.21% DF deferoxamine methane sulfonate (DF) salt was prepared in a nitrogen chamber to prevent contact with oxygen and used as a diluent of CMH solution (1-hydroxy-3-methoxycarbonyl-2,2,5,5-tetramethylpyrrolidine, 0.046 mg/mL); 100 mg of tissue was minced with 200 μL of CMH. Sealed samples were incubated at 37 °C and centrifuged at 1200 rpm for 30 min, followed by another centrifugation cycle at 7000 rpm for 10 min. The samples were returned to the nitrogen chamber, sealed, and read in triplicate in an EPR system (E. Scan EPR Bruker, Massachusetts, USA) [25].

3.11 Statistical analysis

Data are reported as mean ± SEM. Two-Way Analysis of Variance (ANOVA) followed by the Student-Newman-Keuls post-hoc test was used for comparisons between groups. Statistical significance was established at $p \leq 0.05$.

4. Results

4.1 Effectiveness of ovariectomy

To confirm the effects of ovariectomy, we weighted the uterus of all experimental animals (Table 1). Atrophy in the OVX group was confirmed by the absence of sex hormones after removal of the ovaries.

Table 1. Uterus weight index.

Group	Weight index (%)
OVX	0.04 ± 0.006
proestrus	0.18 ± 0.05*
estrus	0.21 ± 0.01*

Uterus weight index was higher in proestrus-sham and estrus-sham *vs* OVX-sham females (* $p < 0.01$ vs OVX). Results are the mean ± S.E. (n = 7). One-Way Analysis of Variance (ANOVA) followed by the Student-Newman-Keuls post-hoc test was used for comparisons between groups.

4.2 Estradiol during proestrus stage diminishes the infarcted area

To study the effect of the presence or absence of sex hormones at the time of MI, 17-β estradiol and progesterone levels were measured to confirm the serum hormone peaks at the time when CO was performed and at 48 h or two weeks after CO. We observed higher 17-β estradiol levels in the proestrus-sham group vs OVX and estrus groups (Fig. 1), while progesterone was higher in the estrus sham vs OVX and proestrus groups (Fig. 1). As we expected, 48 h and 2 weeks after CO, the levels of both hormones were higher in the estrus and proestrus groups than in OVX animals. analyzed the animal's hearts, measuring the infarcted area 48 hours and two weeks after CO. Fig. 1 shows the results of the infarcted area. We found that the groups submitted to CO during the proestrus phase had a smaller infarction area after 48 h and two weeks compared to the OVX and estrus groups, regardless of whether or not they had a regular estrous cycle. We found no difference between CO + OVX females vs those occluded during estrus. We then looked for morphological changes induced by MI in the cardiac chambers and the effect of sex hormones on these changes. Table 2 shows the weight index of the heart chambers weight index. No significant differences were found between groups.

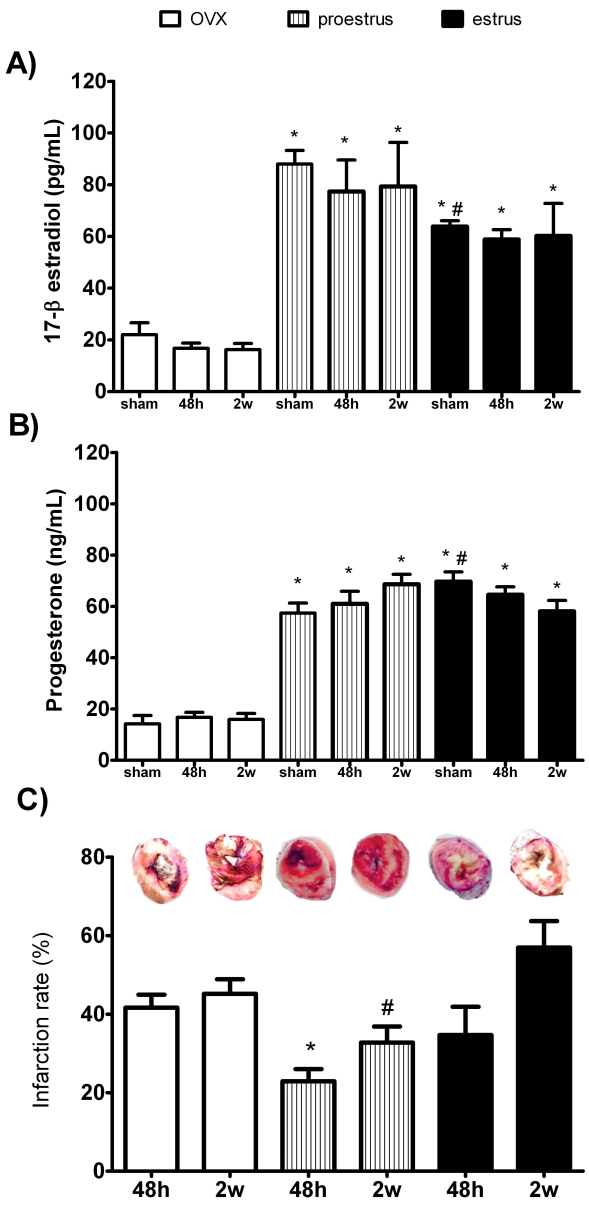

Fig. 1. Estradiol during proestrus diminishes the infarcted area. (A) 17-β estradiol levels. Proestrus animals showed higher estradiol levels vs OVX (* $p < 0.01$); estrus-sham animals showed a decrease in estradiol vs proestrus-sham (# $p < 0.05$). (B) Progesterone levels. Proestrus and estrus groups showed higher progesterone levels vs OVX (* $p < 0.01$); estrus-sham animals showed an increase in progesterone vs proestrus-sham (# $p < 0.05$). (C) Infarction rate. The myocardial infarction area was smaller in the proestrus groups compared to OVX and estrus (* $p < 0.01$ vs OVX-48 h CO; # $p < 0.05$ vs estrus-2 w CO). Data are reported as mean ± S.E. Two-Way Analysis of Variance (ANOVA) followed by the Student-Newman-Keuls post-hoc test was used for comparisons between groups.

4.3 Sex hormones attenuate hemodynamic changes after myocardial infarction

We also measured hemodynamic parameters (systolic and diastolic blood pressure, LVSP, LVDP, HR, and ± dP/d*t*), that could shed light on the functional changes that

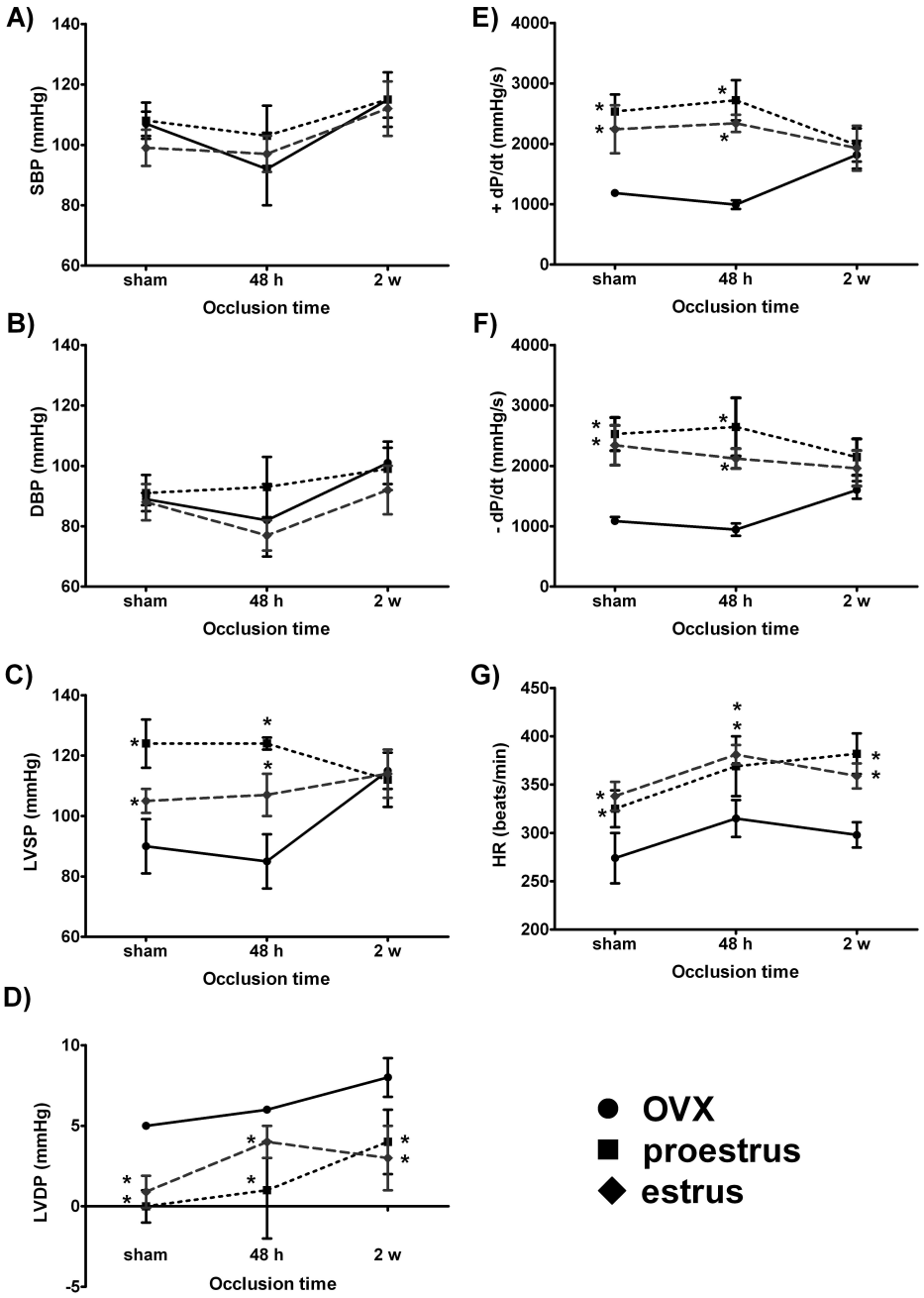

Fig. 2. Sex hormones attenuate hemodynamic changes after myocardial infarction. (A) No differences were found in systolic blood pressur (SBP). (B) No differences were found diastolic blood pressure (DBP). (C) Left ventricular systolic pressure (LVSP) was higher in the proestrus and estrus groups vs OVX (* $p < 0.05$). (D) Left ventricular diastolic pressure (LVDP) was higher in the OVX groups (* $p < 0.05$). (E) + dP/d*t* was higher in the proestrus and estrus groups than in the OVX-sham and OVX-48 h CO groups (* $p < 0.05$). (F) –dP/d*t* was higher in the proestrus and estrus groups than in the OVX-sham and OVX-48 h CO groups (* $p < 0.05$). (G) Heart rate (HR) was lower in the OVX groups (* $p < 0.05$). Values are the mean ± S.E. Two-Way Analysis of Variance (ANOVA) followed by the Student-Newman-Keuls post-hoc test was used for comparisons between groups.

occur after MI and on the effect of sex hormones. Fig. 2 shows the results obtained by cardiac catheterization. No significant difference was found in the SBP or DBP between the estrus, proestrus and OVX groups at 48 h or 2 weeks after CO. However, OVX-sham females, however, showed a significant decrease in HR, probably due to sex hormones depletion. Interestingly, higher HR values were observed in the estrus and proestrus groups compared to the respective OVX group. These values also tended to be higher in the CO groups at both times. On the other hand, LVSP increased in the OVX-2 w CO vs OVX-sham group but was higher in the estrus and proestrus sham and 48 h of CO vs OVX. Regarding LVDP, there was a significant increase in the OVX females groups when com-

Table 2. Heart chambers weight index.

Group	Oclussion time	Cardiac Chambers (mg/g)			
		LV	RV	LA	RA
OVX	sham	1.70 ± 0.09	0.86 ± 0.08	0.09 ± 0.01	0.07 ± 0.01
	48 h	1.79 ± 0.18	0.56 ± 0.04	0.11 ± 0.01	0.06 ± 0.01
	2 w	1.43 ± 0.05	0.91 ± 0.03	0.09 ± 0.00	0.06 ± 0.00
Proestrus	sham	1.62 ± 0.10	1.01 ± 0.08	0.11 ± 0.01	0.07 ± 0.01
	48 h	1.88 ± 0.14	0.83 ± 0.02	0.11 ± 0.02	0.07 ± 0.01
	2 w	1.87 ± 0.09	0.88 ± 0.04	0.12 ± 0.01	0.08 ± 0.01
Estrus	sham	1.65 ± 0.09	0.81 ± 0.06	0.11 ± 0.01	0.07 ± 0.01
	48 h	1.76 ± 0.11	0.82 ± 0.03	0.10 ± 0.02	0.07 ± 0.01
	2 w	1.74 ± 0.13	0.98 ± 0.18	0.12 ± 0.01	0.08 ± 0.02

No differences were found in heart chambers weight index These results are the mean ± S.E. (n = 7). VI left ventricle; RV right ventricle; AI left atrium; AD right atrium. Two-Way Analysis of Variance (ANOVA) followed by the Student-Newman-Keuls post-hoc test was used for comparisons between groups.

pared to the estrus-sham and proestrus-sham groups, 48 h and two weeks after CO, respectively. When we compared the proestrus and estrus groups with their respective OVX group, we found higher ± dP/d*t* values in the hormone-preserved groups.

4.4 Effects of sex hormones on NO levels after MI

When we quantified NO in samples obtained from the left ventricular penumbra (Fig. 3), we detected lower levels of NO in the OVX-2 w group compared to the OVX-sham group. No changes were found in the proestrus groups. The estrus-2 w CO group had higher levels than the estrus-sham group. On the other hand, the estrus and proestrus groups showed lower concentrations of NO than the OVX-sham and OVX-48 h groups, respectively. NO production was higher in the estrus and OVX groups at 2 weeks after CO, vs proestrus-2 w CO.

4.5 The absence of sex hormones increases ROS levels after myocardial infarction

Finally, to relate the loss of hormones to oxidative stress, we quantified ROS in left ventricular penumbra samples (Fig. 4). OVX females showed a significant increase in ROS after 48 h and two weeks after CO compared to OVX-sham animals. Among the infarcted females in the proestrus and estrus phases, there were no significant differences in ROS levels along MI evolution. In the sham groups, lower levels of ROS were quantified in ovx females compared to females in the estrous and proestrus phases. In the 48 h CO groups, lower levels of ROS were observed in infarcted females in the estrus phase compared to females in the proestrus phase and OVX. there were no significant differences in the levels of ROS between groups at 2 weeks after CO.

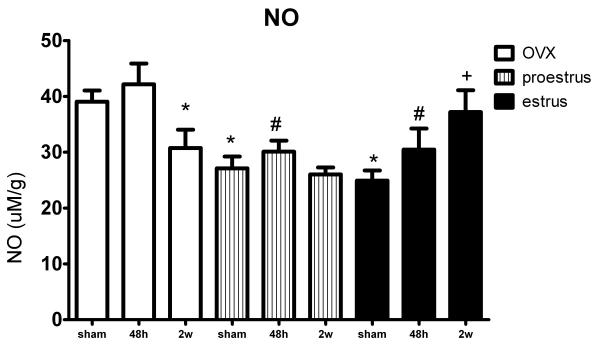

Fig. 3. Effects of sex hormones on NO levels after myocardial infarction. NO levels were lower in proestrus (* $p < 0.01$ vs OVX-sham; # $p < 0.05$ vs OVX-48 h). On the other hand, NO levels were higher in estrus-2 w CO than estrus-sham (+ $p < 0.05$). Results are the mean ± S.E. Two-Way Analysis of Variance (ANOVA) followed by the Student-Newman-Keuls post-hoc test was used for comparisons between groups.

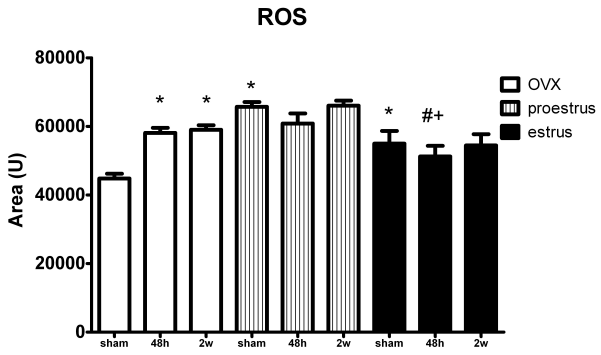

Fig. 4. The absence of sex hormones increases ROS levels after myocardial infarction. Oxidative stress was higher in OVX after MI (* $p < 0.01$ vs OVX-sham). No differences were found in the proestrus or estrus groups after MI. ROS levels were lower in estrus-48 h CO (# $p < 0.05$ vs OVX-48 h CO; + $p < 0.05$ vs proestrus-48 h CO). Values are the mean ± S.E. Two-Way Analysis of Variance (ANOVA) followed by the Student-Newman-Keuls post-hoc test was used for comparisons between groups.

5. Discussion

In this work we found that the extent of initial damage caused by CO, seems to be related to hormonal levels at the time of infarction. The cardioprotective effects of estrogens have been widely reported [26–28], nevertheless, previous studies that compared the incidence of MI between premenopausal and postmenopausal women only considered the presence and absence of estrogens as a determining factor [29, 30]. However, the influence of variations in this hormone throughout the estrous cycle has not been studied in acute or chronic MI. In this work we demonstrate the importance of estrogens variations during the post-infarction adaptive period. Our results are in agreement with Mukamal *et al*. [6] who, two decades ago, reported a threefold higher risk of MI during the early follicular phase in women when estrogen and progesterone levels are lower. However, the authors did not further investigate the underlying mechanisms or post-MI cardiovascular function. In this regard, we demonstrated that the estrous cycle phase during which the injury occurs, determines the severity of disease evolution. We propose that estrogens in the proestrus stage [31, 32], confer cardioprotection by reducing the infarction area, in addition to maintaining the contractile capacity of the left ventricle and preventing changes in NO and ROS levels.

Some investigations suggest that estrogens reduce decrease the infarction area [15, 33, 34]. However, most of these studies involved exogenous administration of hormones. In this work, we showed that physiological concentrations of sex hormones during the proestrus phase may help to protect the heart during the infarction process. The cardioprotective mechanisms are diverse. Pelzer *et al*. [35], demonstrated *in vitro* that estrogens inhibit apoptosis in cardiomyocytes, which may be related to a decrease in the activation of caspase 3, a protein involved in this process [36]. As demonstrated with the use of a specific ERβ agonist, the activation of Erβ is essential for the improvement of cardiac recovery after the ischemia/reperfusion (I/R) process through the inhibition of apoptosis and preservation of mitochondrial integrity. Also, E2 modulates MLC2 activation after I/R independently of the presence of ERβ presence [37]. Interestingly, among a wide variety of proposed mechanisms, most of them converge to the preservation of mitochondrial function and structure [38]. Zhou *et al*. [39] found that ERα promotes β-cell survival and insulin secretion through maintenance of mitochondrial fission/fusion-mitophagy dynamics and endoplasmic reticulum function, in part mediated by Oma1 and Chop repression.

It is worth to mentioning that, in our study, none of the groups developed cardiac hypertrophy as a consequence of MI. Some authors have found an increased deposition of collagen in the myocardium after infarction in OVX females when the post-infarction period was longer than ten weeks [40]. We believe that the absence of cardiac hypertrophy is related to the systemic pressure values that are maintained despite the infarction. Our results agree with some researchers who have found no change in mean arterial pressure or systemic pressure at 2 or 6 weeks post-MI in OVX and non-OVX females [41]. On the other hand, the lower HR in the OVX groups indicates that the loss of ovarian hormones has a direct impact on this parameter. Jankowski *et al*. [42] 2001 reported a decrease of atrial natriuretic peptide in OVX rats that was associated with a reduction in HR. Supporting our results, Pinkham *et al*. [41] found a lower HR in OVX females with and without infarction compared to those with intact ovaries. Also, females with MI that received exogenous 17-β estradiol showed higher HR [43]. These findings could be related to increased catecholamines reactivity in OVX rats after infarction [44]. In fact, it has been previously reported that the regulation of the sympathetic nervous system is altered in females, including attenuation of sympathoadrenal activation and augmentation of sympathoadrenal inhibition [45].

Moreover, we found lower LVSP and higher LVDP values in the OVX groups. Within this context, Wan *et al*. [46] in 2014, demonstrated that the increase in LVDP is an important early indicator of heart failure in humans. The rise in LVDP after MI may indicate a decreased relaxation ability of the heart during ventricular filling. In addition to ventricular pressures, relaxation and contraction indexes expressed as –dP/d*t* and +dP/d*t*, respectively, allow to estimate the contractile capacity of the ventricle [47]. Van Eickels in 2003 and Almeida in 2014 reported a decrease in the $\pm$dP/d*t* index after heart attack and OVX [36, 40], in agreement with our work. Isovolumometric relaxation (–dP/d*t*) is an energy-dependent process in which calcium ions exit the cytoplasm against a concentration gradient, allowing dissociation of the actin-myosin contractile complex [48]. Thus, the alteration in calcium homeostasis directly influences cardiac contraction and relaxation. In this process, the calcium reuptake from the cytosol into the sarcoplasmic reticulum is mediated by SERCA2a in the presence of ATP [49]. Some studies have described that estrogen depletion decreases the expression and activity of both SERCA2a and ATPase, reducing Ca^{2+} reuptake in cardiomyocytes [50]; however, the administration of estradiol can prevent intracellular calcium changes and improve myocardial relaxation in OVX females [51].

Silberman *et al*. [52], in 2010, have associated the effect of MI on ventricular relaxation with oxidative stress and reduced activity/production of NOS/NO, suggesting that oxidative stress is a result of NOS decoupling. Previous investigations indicated that estradiol upregulates the expression and activity of eNOS in endothelial cells [14, 53]. Higher levels of estradiol increase NO production and represent one of the most important cardioprotective mechanisms at the vascular level in pathologies such as heart attack [54]. However, we obtained different re-

sults, NO levels were not modified in the proestrus phase but were higher in OVX females, suggesting that NO may exert different effects on the heart according to the enzyme that produces it. Future research is required to confirm this suggestion.

Sheng-an *et al*. [55], in 2016, have associated iNOS with the development of inflammation in MI and observed an increase in NO. Thus, the NO found in cardiac tissue may have been generated by iNOS in OVX females. Some studies suggest that inflammatory factors can increase the infarction area and LVDP, reduce LVSP, and promote ventricular remodeling, causing heart failure [56, 57]. Furthermore, Zancan *et al*. [58] demonstrated that 17-β estradiol, but not progesterone, decreases the expression and activity of iNOS in rat aortic smooth muscle cells. This would explain our finding of lower NO levels during the proestrus phase compared to OVX females. Interestingly, there were no changes in NO levels in females in proestrus, although the infarcted area was smaller. Wang *et al*. [59], in 2018, demonstrated an increase of NO production in OVX rats and also found overexpression of iNOS, which would agree with our results. Therefore, we suggest NO to be an inflammatory biomarker under these conditions.

On the other hand, it is known that, during infarction, oxygen deprivation inhibits electron flow in mitochondria, rendering ATP use inefficient and turning ATP synthase into an ATPase that consumes ATP to extrude protons from the matrix to the intermembrane space [60, 61]. When ischemia is permanent, the Na^+/K^+ ATPase is inhibited and the cellular acidification induced by lactic acid accumulation activates the Na^+/H^+ exchanger in an attempt to restore cellular pH. This, in turn, causes an increase in intracellular Na^+ that activates the Na^+/Ca^{2+} exchanger, which leads to Ca^{2+} overload [62]. The elevation of Ca^{2+} can induce opening of the mitochondrial transition pore (PTPm) and the release of ROS [63].

Based on our results, we propose that oxidative stress is controlled if CO occurs in the presence of sex hormones. Feng *et al*. [64] showed that acute treatment with estradiol was able to close the PTPm through its GPER receptor, activating the MERK/ERK signaling pathway that phosphorylates GSK-3β, deactivating this kinase and reducing mitophagy. The ubiquitination of mitochondrial proteins and Ca^{2+} overload increase the concentrations of this ion, inducing PTPm opening. These phenomena reduce the generation of ROS, with subsequent reduction of the infarcted area, as we showed here.

In 2016, Luo *et al*. [65] reported a relationship between the expression of estrogen receptors and the decrease in oxidative stress and infarcted area. The authors attributed these effects to the upregulation of p38β, with a consequent increase in its activity, phosphorylating Mn-SOD, which resulted in the suppression of ROS and decreased the infarcted area, as we observed in this work. Besides, Rizzo *et al*. [66], in 2009, demonstrated higher physiological levels of ROS in the proestrus and estrus phases, which are related to the acute inflammatory process that occurs during the follicular and ovulatory phases in female dogs, in agreement with our results obtained for the groups not submitted to CO.

6. Conclusions

The phase of the estrous cycle in which myocardial infarction occurs is important. When coronary occlusion occurs during proestrus, it prevents changes in cardiac function, the development of hypertrophy, oxidative stress and changes in NO levels, and reduces the extent of infarction.

7. Perspectives

Our work showed that ovarian hormones influence cardiac function in young Wistar rats; nevertheless, it is necessary to analyze the changes in ovarian hormones that occur during aging and how estrogen replacement therapy can improve survival in post-menopausal women. This could help to emphasize the importance of considering hormonal influences on cardiovascular diseases and encourage scientists to investigate sex differences in the causes, diagnosis, pathophysiology and treatments in this field.

8. Author contributions

JFM, DRH and PLS conceived and designed the experiments; DRH performed the experiments; JFM, DRH analyzed the data; SFC, MCRH contributed reagents and materials.

9. Ethics approval and consent to participate

Local ethics committee approval was obtained with the informed consent of all participants. The institutional review board of the CICUAE-FESC approved the use of animals in this research project, code C18_06.

10. Acknowledgment

The authors thank C. Ramon Martínez-Gomez and Juan Martinez-Pariente from CINVESTAV-IPN Unidad Sur for providing the experimental animals.

11. Funding

This work was supported by grants from PAPIIT IN213318, PAPIIT IA205119, PIAPI 1828-FES Cuautitlan, Universidad Nacional Autonoma de México and CONACYT A1-S-8958.

12. Conflict of interest

The authors declare no conflict of interest.

13. References

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Keywords: Estrous cycle; Reactive oxygen species; Nitric oxide; Myocardial infarction

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