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# Sex differences in cardiac dynamics during myocardial ischemia using a single cell approach

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**Myocardial ischemia, arising from severe blockages in coronary arteries, poses a significant global health risk due to its potential to cause arrhythmia and heart failure, often leading to sudden cardiac death. During acute myocardial ischemia, profound changes occur in cardiac electrophysiology and anatomy, influencing action potential morphology and propagation, which increased susceptibility to arrhythmias. Sex differences play a critical role in myocardial ischemia and arrhythmogenesis. Females exhibit distinct genetic and hormonal influences on ion channel expression and cardiac function, affecting susceptibility to arrhythmias like Torsade de Pointes. Using the O'Hara-Rudy dynamic (ORd) model, this study shows that females are more likely than males to exhibit cardiac alternans (2:2), a periodic variation in action potential duration between consecutive heartbeats, as well as 2:1 arrhythmic behaviors-characterized by inexcitability in the even beats-under ischemic conditions. Additionally, hormones further exacerbate these gender differences. Moreover, females show a higher propensity than males to terminate 2:2 and 2:1 arrhythmic responses during ischemia treatment. This manuscript aims to uncover sex-specific disparities in electrophysiological responses and drug reactions during myocardial ischemia using the optimized ORd model. These findings underscore the importance of considering sex-specific factors in cardiovascular research and clinical practice.**

Myocardial ischemia arises from severe blockages in coronary arteries, potentially leading to serious complications such as heart attack, arrhythmia, and heart failure, making it a primary cause of sudden cardiac death worldwide[1–4]. During acute myocardial ischemia, significant changes occur in cardiac electrophysiology and anatomy[5–8], influencing the morphology and propagation of action potentials. Specifically, within the first 10–15 min of ischemia, significant changes in membrane potential occur due to impaired ion channel function and disrupted voltage and concentration gradients. These changes lead to a shortened action potential duration (APD), a prolonged effective refractory period (ERP), reduced conduction velocity (CV), and an increased susceptibility to ectopic beats and triggered activity.

The variations in electrophysiological properties between normal and ischemic tissue form the foundation for reentrant arrhythmias, as observed in both experimental and simulation research[5,9–11]. Previous studies have identified four primary components of regional acute ischemia lasting 10–15 min: hyperkalemia, acidosis, anoxia, and gap junction remodeling. Hyperkalemia is characterized by increasing extracellular potassium concentration ($[K^+]_o$)[12,13], resulting in elevated cell resting membrane potential and reduced cell excitability. Acidosis, characterized by low pH levels[14], impacts the conductance and kinetics of fast sodium (INa)[15] and L-type calcium (ICaL) currents[16]. Anoxia, caused by low intracellular ATP levels[17], activates ATP-dependent potassium ($I_{\mathrm{Katp}}$)[18] and affects ATP-dependent ICaL currents. Additionally, ischemia induces gap junctional uncoupling and remodeling in cardiac tissue[19,20]. Furthermore, the "finger-like" geometry of ischemic regions penetrating into normal tissue should also be considered as another characteristic of myocardial ischemia[8].

In addition to the electrophysiological and anatomical factors underlying cardiac arrhythmias, female gender is another key factor influencing both inherited and acquired long-QT syndrome, which is associated with Torsade de Pointes (TdP) arrhythmias[21–26]. Research has demonstrated genomic differences in ion channel expression between males and females, along with sex-specific steroid hormones that influence the modulation of these ion channels[22,23,25–29]. Moreover, recent clinical and experimental studies suggest that variations in susceptibility to certain arrhythmias may result from these inherent sex-based differences in cardiac tissue[30–33]. Investigations into sex differences in myocardial ischemia[34–37] have highlighted the importance of developing sex-specific therapeutic strategies. However, the precise disparities in myocardial ischemia and differences in drug responses between sexes remain poorly understood.

Cardiac alternans is characterized as a 2:2 response, where a periodic alteration in APD occurs, with each pair of stimuli inducing alternating short and long action potentials during rapid, periodic pacing. This

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phenomenon reflects an inherent instability within the cardiac conduction system and can signal an increased risk of arrhythmias. In contrast, a 1:1 response describes a stable condition where each pacing stimulus generates a consistent action potential with no alternans, ensuring a reliable cardiac action potential. Alternatively, a 2:1 response occurs when every two pacing stimuli result in only one action potential, meaning that the even-numbered beats do not generate an action potential, potentially indicating a conduction block or impairment in the heart's electrical responsiveness. During myocardial ischemia, fluctuations in repolarization dynamics can lead to alternans, increasing the risk of arrhythmias and impaired cardiac function. Additionally, the metabolic and ionic disturbances caused by ischemia compromise the heart's ability to efficiently propagate electrical impulses, which can result in 2:2 and 2:1 responses. Both response patterns highlight significant changes in cardiac electrical activity during ischemia, emphasizing the heightened susceptibility to arrhythmias and the critical need for monitoring and intervention in affected patients.

In this manuscript, the aim is to uncover sex-specific disparities in electrophysiological properties and drug responses during myocardial ischemia using the optimized O'Hara–Rudy dynamic (ORd) model. The major findings highlight that females exhibit more frequent 2:2 and 2:1 behaviors under ischemic conditions, and the influence of hormones further enhances these differences. Additionally, females are more likely than males to terminate 2:2 responses during myocardial ischemia treatment. These insights underscore the importance of considering sex-specific factors in cardiovascular research and clinical practice, potentially leading to tailored therapeutic strategies that improve outcomes and reduce disparities in heart disease treatment between men and women.

## Results

### Sex difference in APD during myocardial ischemia

In this section, the aim is to demonstrate the gender differences in electrophysiological properties during myocardial ischemia and the subsequent responses to medication administration for the ischemic condition.

Figure 1 focuses on APD of the last two beats ($\mathrm{APD}_{\max}$, $\mathrm{APD}_{\min}$) and their difference ($\mathrm{APD}_{\mathrm{diff}}$), serving to quantify 2:2 pattern during steady state at a BCL of 300 ms. This investigation encompasses four scenarios: males endocardium cells without hormone treatment (A), males endocardium cells with 35nM DHT (B), females endocardium cells without hormone treatment (C), and females endocardium cells in the late follicular phase (D), under ischemic conditions governed by $f_{\mathrm{Katp}}$ and $[\mathrm{K}^+]_{\mathrm{o}}$. Figure 1 demonstrates that APD decreases with increasing $f_{\mathrm{Katp}}$ while $[\mathrm{K}^+]_{\mathrm{o}}$ remains fixed. On the other hand, with constant $f_{\mathrm{Katp}}$ levels, an increase in $[\mathrm{K}^+]_{\mathrm{o}}$ generally leads to a slight increase in APD. Notably, females in the late follicular phase exhibit a biphasic APD response when $f_{\mathrm{Katp}}$ is equal to 0. Specifically, a prolonged APD was observed when $[\mathrm{K}^+]_{\mathrm{o}}$ reached extreme values, which is attributed to the presence of a 2:1 pattern. Moreover, as shown by the $\mathrm{APD}_{\mathrm{diff}}$, 2:2 behaviors are observed across all four scenarios when $[\mathrm{K}^+]_{\mathrm{o}}$ falls within the intermediate range, irrespective of $f_{\mathrm{Katp}}$ levels.

When examining sex differences during myocardial ischemia, females exhibit a more pronounced decrease in APD with increasing $f_{\mathrm{Katp}}$ while $[\mathrm{K}^+]_{\mathrm{o}}$ remains fixed. Additionally, females without hormones show a higher propensity for 2:2 pattern compared to males without hormones. However, the presence of 35 nM DHT in males diminishes this difference, whereas females with hormones show an even larger disparity compared to males. Specifically, males treated with 35 nM DHT show a slight increase in the occurrence of the 2:2 pattern and shift the regions where this pattern occurs compared to males without hormone. Similarly, females in the late follicular phase exhibit heightened likelihood of 2:2 and 2:1 behaviors. Sex-specific trends in APD are also observed in the epicardium (data not shown), though 2:2 behaviors are not present.

Cardiac alternans occur due to rapid periodic pacing, which disrupts the normal repolarization of the action potential from the previous beat. This disruption causes the action potential to exhibit a long-short or short-long duration pattern. Additionally, ionic modulation during repolarization can make cardiac myocytes more susceptible to alternans, as altered calcium or potassium handling can influence contractility and electrical stability. Compared to males, the female ventricular cardiac cell model exhibits a reduced repolarizing potassium current and an increased calcium current, leading to altered repolarization kinetics. This results in a longer APD and heightened susceptibility to cardiac alternans under ischemic conditions. Additionally, estrogen further reduces the activity of IKr compared to the control, leading to increased variability in action potentials and contributing to the development of alternans.

### Sex difference in electrophysiology during myocardial ischemia

To gain a more comprehensive understanding of the sex-specific differences in electrophysiological behaviors, the voltage, concentration and current traces under conditions of no ischemia ($f_{\mathrm{Katp}} = 0$, $[\mathrm{K}^+]_{\mathrm{o}} = 4$ mM), moderate ischemia ($f_{\mathrm{Katp}} = 0$, $[\mathrm{K}^+]_{\mathrm{o}} = 6$ mM and $f_{\mathrm{Katp}} = 0.1$, $[\mathrm{K}^+]_{\mathrm{o}} = 6.5$ mM) and severe ischemia ($f_{\mathrm{Katp}} = 0.2$, $[\mathrm{K}^+]_{\mathrm{o}} = 9$ mM) were analyzed, as depicted in Figs. 2, 3, 4 and 5, respectively. Specifically, the temporal data of voltage, intracellular calcium concentration ($[\mathrm{Ca}]_i$), and key currents including ICaL, Ito, IKr, IKs, IK1, IKatp, and INaCa were presented.

As shown in Fig. 2, under non-ischemic conditions, the differences in electrophysiological variables between females (red solid) and males (blue solid) without hormones are relatively minor. Specifically, females show a larger APD but smaller amplitude of $[\mathrm{Ca}]_i$, IKr, IKs and IK1 compared to males. However, these differences become more pronounced when hormones are involved. Specifically, females in the late follicular phase (red dashed line) exhibited a 2:1 pattern, resulting in a notable increase in APD. Additionally, $[\mathrm{Ca}]_i$ and key currents also exhibited a 2:1 behavior, characterized by smaller amplitude but longer duration compared to males treated with 35nM DHT.

As shown in Fig. 3, during moderate ischemic conditions, the disparities in electrophysiological variables between females (red solid) and males (blue solid) without hormones are evident but slight. Specifically, females and males exhibit in-phase oscillations, where $[\mathrm{Ca}]_i$ alternans are more pronounced than APD alternans.

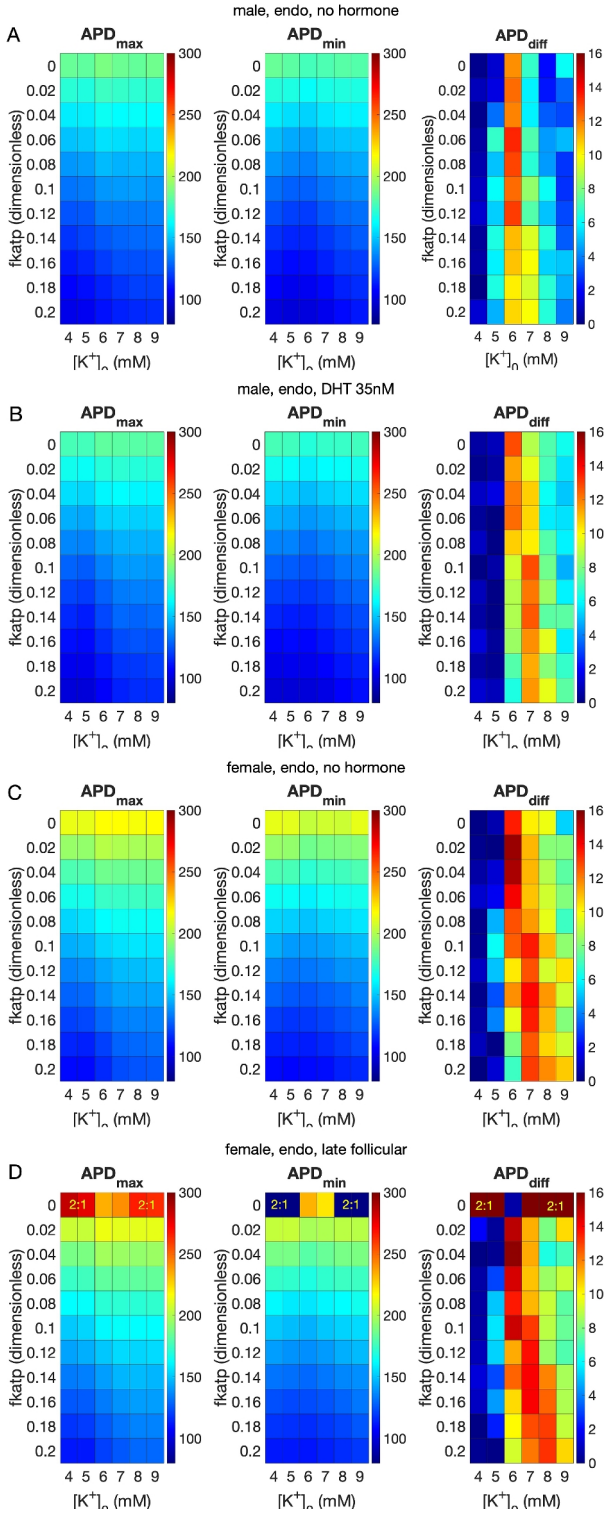


**Fig. 1**. APD ($\mathrm{APD}_{\mathrm{max}}$, $\mathrm{APD}_{\mathrm{min}}$, and $\mathrm{APD}_{\mathrm{diff}}$) of endocardium cells for males without hormone (**A**), males with DHT 35 mM (**B**), females without hormone (**C**), and females in the late follicular phase (**D**) are plotted against $f_{\mathrm{katp}}$ and $[\mathrm{K}^+]_{\mathrm{o}}$, with a fixed BCL of 300 ms.

Additionally, females show a larger amplitude of APD alternans and a smaller amplitude of $[\mathrm{Ca}]_i$ alternans compared to males. However, when hormones are involved, the contrast between males with 35 nM DHT (blue dashed) and females in the late follicular phase (red dashed) becomes pronounced. Specifically, females exhibit a 1:1 behavior, while males show a 2:2 pattern. Furthermore, females show increased APD, along with significantly reduced $[\mathrm{Ca}]_i$, IKr, IK1 and INaCa compared to males.

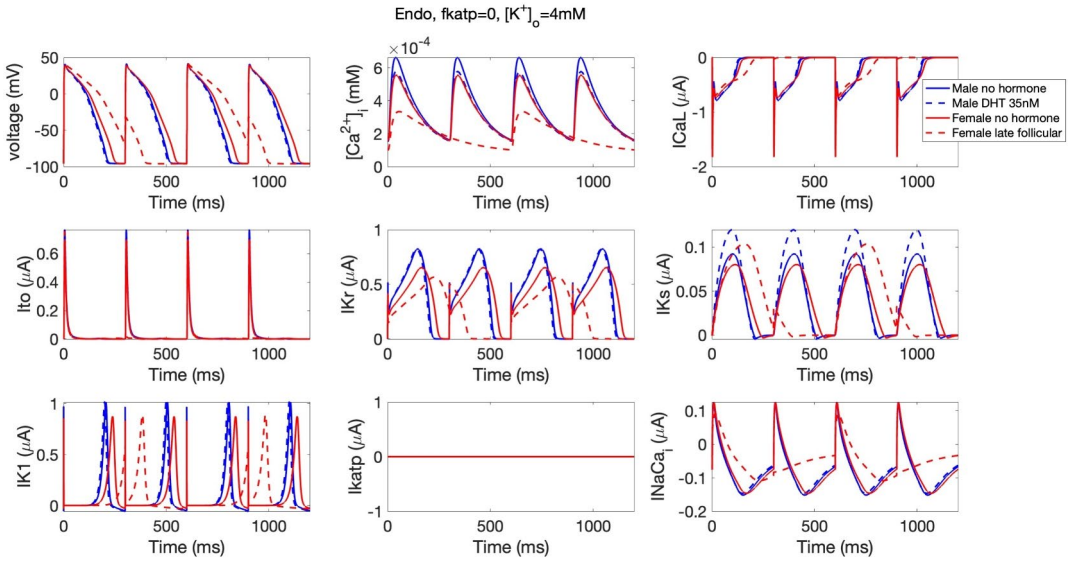


**Fig. 2**. Time-evolution profiles of voltage and ionic currents in the endocardium during myocardial ischemia for males without hormone (blue), males with DHT 35nM (blue dashed), females without hormone (red), and females in the late follicular phase (red dashed), with a fixed BCL of 300 ms, where $f_{\mathrm{katp}} = 0$ and $[\mathrm{K}^+]_{\mathrm{o}} = 4$ mM.

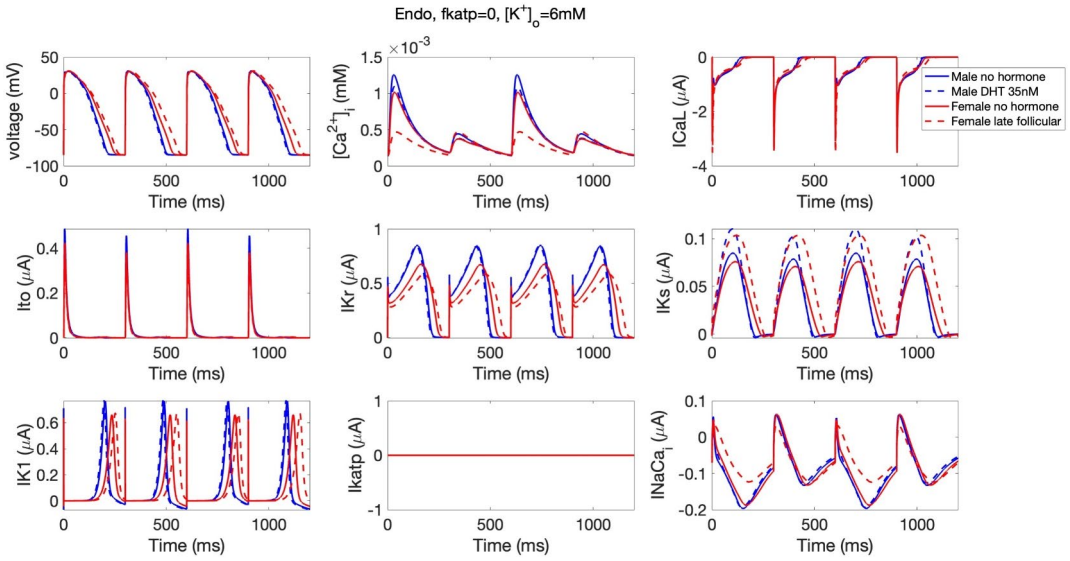


**Fig. 3**. Time-evolution profiles of voltage and ionic currents in the endocardium during myocardial ischemia for males without hormone (blue), males with DHT 35 nM (blue dashed), females without hormone (red), and females in the late follicular phase (red dashed), with a fixed BCL of 300 ms, where $f_{\mathrm{katp}} = 0$ and $[\mathrm{K}^+]_{\mathrm{o}} = 6$ mM.

Figure 4 illustrates another scenario of moderate ischemia where both $f_{\mathrm{Katp}}$ and $[\mathrm{K}^+]_{\mathrm{o}}$ deviate from their nominal values. As shown in the figure, the sex differences in electrophysiological behaviors are similar to Fig. 3. In particular, male and female oscillate in phase without hormones, $[\mathrm{Ca}]_i$ alternans are more significant than APD alternans. However, when hormones are involved, sex differences in APD, IKr, and IKs are heightened, whereas sex specific differences in $[\mathrm{Ca}]_i$ and INaCa are minimized.

Figure 5 depicts the electrophysiological characteristics of both males (blue) and females (red) under severe ischemic conditions. As illustrated, calcium alternans is more pronounced than APD alternans in both sexes. Furthermore, females without hormones (red solid line) exhibit out-of-phase oscillations compared to males with hormones (blue solid line). In particular, females (solid red) exhibit a long-short pattern, while males (solid blue) show a short-long pattern. Additionally, females demonstrate more significant alternans in APD, $[\mathrm{Ca}]_i$, and INaCa. When hormones are involved, the phase of alternans is reversed for both sexes. The difference in

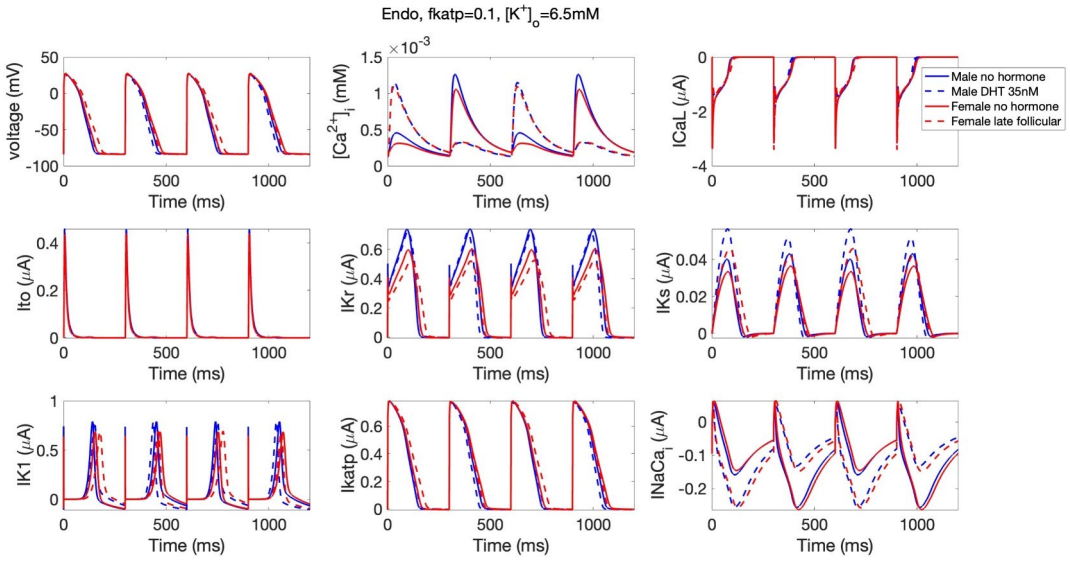


**Fig. 4**. Time-evolution profiles of voltage and ionic currents in the endocardium during myocardial ischemia for males without hormone (blue), males with DHT 35 nM (blue dashed), females without hormone (red), and females in the late follicular phase (red dashed), with a fixed BCL of 300 ms, where $f_{\mathrm{katp}} = 0.1$ and $[\mathrm{K}^+]_{\mathrm{o}} = 6.5$ mM.

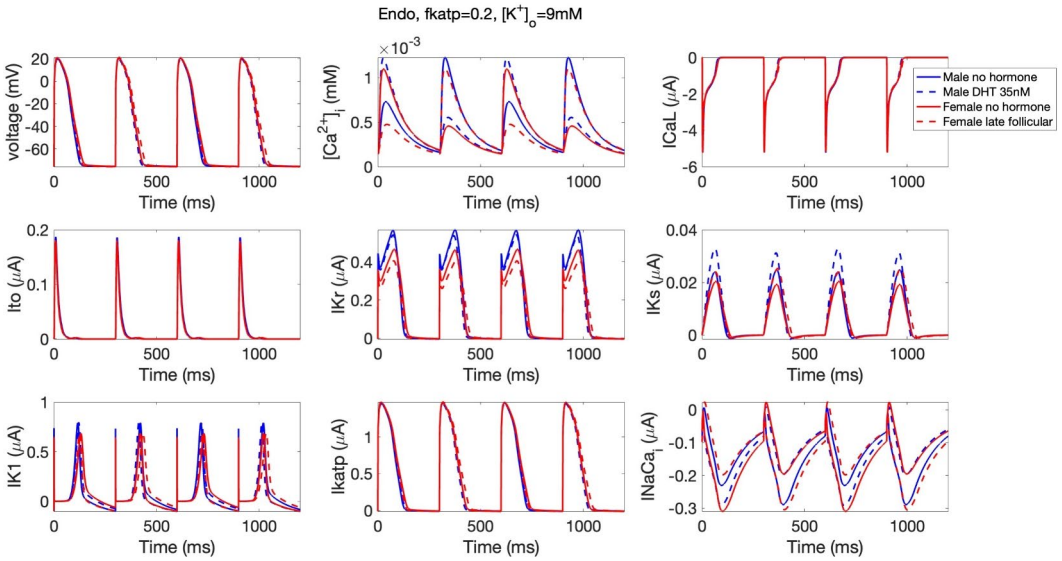


**Fig. 5**. Time-evolution profiles of voltage and ionic currents in the endocardium during myocardial ischemia for males without hormone (blue), males with DHT 35 nM (blue dashed), females without hormone (red), and females in the late follicular phase (red dashed), with a fixed BCL of 300 ms, where $f_{\mathrm{katp}} = 0.2$ and $[\mathrm{K}^+]_{\mathrm{o}} = 9$ mM.

alternans between females in the late follicular phase and males treated with 35nM DHT is minimal. However, females still exhibit lower IKr, IKs, and IK1 compared to males.

Figures 3, 4 and 5 demonstrate that the divergence in the severity of calcium versus voltage alternans during different ischemic states can be attributed to the intricate relationships between action potential dynamics and calcium handling. Under moderate and severe ischemia, the dysregulation of calcium release and reuptake processes significantly enhances calcium alternans, while voltage alternans, affected to a lesser extent, do not show similar levels of variability. Additionally, functional sex differences in $\mathrm{Ca}^{2+}$ handling also play a crucial role in this context.

## Sex difference in drug responses during myocardial ischemia

In this section, the sex-specific differences in drug responses during the severe ischemia were investigated, characterized by $f_{\mathrm{Katp}} = 0.2$ and $[\mathrm{K}^+]_{\mathrm{o}} = 9$ mM. In particular, In particular, the 14 medications were assessed and categorized into three classes based on their effects in males and females: (1) out-of-phase oscillation between females and males, (2) 1:1 response observed in both sexes and (3) occurrence of 2:2 behavior observed in males only. Therefore, one representative drug from each category was selected, namely Bepridil, Nitrendipine, and Nifedipine, for further analysis. In particular, the temporal dynamics of voltage, $[\mathrm{Ca}]_i$, and key currents (ICaL, Ito, IKr, IKs, IK1, IKatp, and INaCa) following four dose administration of each of these three drugs were plotted.

Figure 6 depicts how voltage, $[\mathrm{Ca}]_i$, and key currents evolve over time after administering four doses of Bepridil. As depicted in this figure, calcium alternans, including $[\mathrm{Ca}]_i$ and INaCa, are more pronounced than APD alternans for both sexes. Interestingly, females and males exhibit opposite behaviors: they oscillate out of phase. Upon hormone administration, the phase of oscillation reverses for both.

Figure 7 illustrates the temporal evolution of voltage, $[\mathrm{Ca}]_i$, and key currents following the administration of four doses of Nitrendipine. As observed in this figure, alternans, except for INaCa, is minimal for both sexes, showing a 1:1 behavior. However, males and females exhibit out-of-phase oscillations in INaCa. Administering hormones increases IKs in both sexes.

Figure 8 depicts the time-dependent changes in voltage, $[\mathrm{Ca}]_i$, and major currents subsequent to the administration of four doses of Nifedipine. As evident from the figure, females do not exhibit alternans except in INaCa, whereas males continue to show alternans. Upon hormone addition, alternans in $[\mathrm{Ca}]_i$ are suppressed in males, with minimal effect observed in females. Additionally, INaCa alternans are reversed in both sexes following hormone administration.

## Discussion

The aim of this study is to explore sex-specific differences in electrophysiological responses and drug effects during myocardial ischemia, using the optimized ORd model. The main findings highlight that females exhibit more frequent 2:2 and 2:1 behaviors under ischemic conditions, and the influence of hormones further enhances these differences. Additionally, females are more likely than males to terminate 2:2 responses during myocardial ischemia treatment. These insights underscore the importance of considering sex-specific factors in cardiovascular research and clinical practice, potentially leading to tailored therapeutic strategies that improve outcomes and reduce disparities in heart disease treatment between men and women.

Myocardial ischemia is significant due to its potential to lead to severe cardiac events such as heart attacks (myocardial infarction) and cardiac arrhythmias[1–4]. Understanding myocardial ischemia is crucial for developing effective treatments to reduce heart damage and improve patient outcomes. Research in this area also helps in identifying risk factors, developing preventive strategies, and advancing diagnostic techniques for cardiovascular diseases. Furthermore, studying myocardial ischemia contributes to the broader understanding of cardiovascular physiology and pathophysiology, aiding in the development of new therapeutic interventions. Recent research findings[38] investigates the effects of ORM-10103, a selective inhibitor of the $\mathrm{Na}^+/\mathrm{Ca}^{2+}$ exchanger (NCX), on intracellular calcium dynamics and cell viability in isolated canine ventricular cardiomyocytes subjected to conditions mimicking ischemia and reperfusion. The study affirms that selective NCX inhibition via ORM-10103 can provide protective effects against pathological alterations in intracellular calcium handling during ischemia/

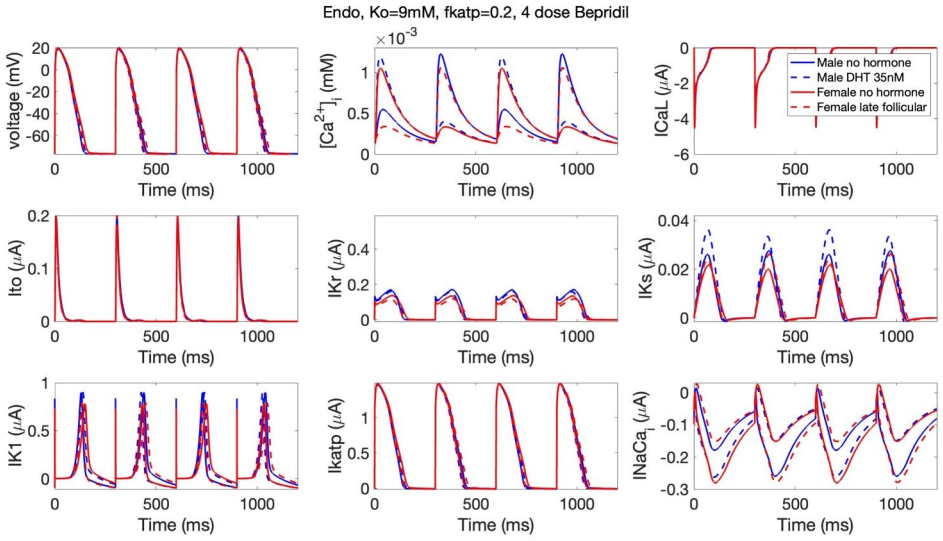


**Fig. 6**. Time-evolution profiles of voltage and ionic currents in the endocardium during myocardial ischemia under the treatment of 4 dose Bepridil for males without hormone (blue), males with DHT 35 nM (blue dashed), females without hormone (red), and females in the late follicular phase (red dashed), with a fixed BCL of 300 ms, where $f_{\mathrm{katp}} = 0.2$ and $[\mathrm{K}^+]_{\mathrm{o}} = 9$ mM.

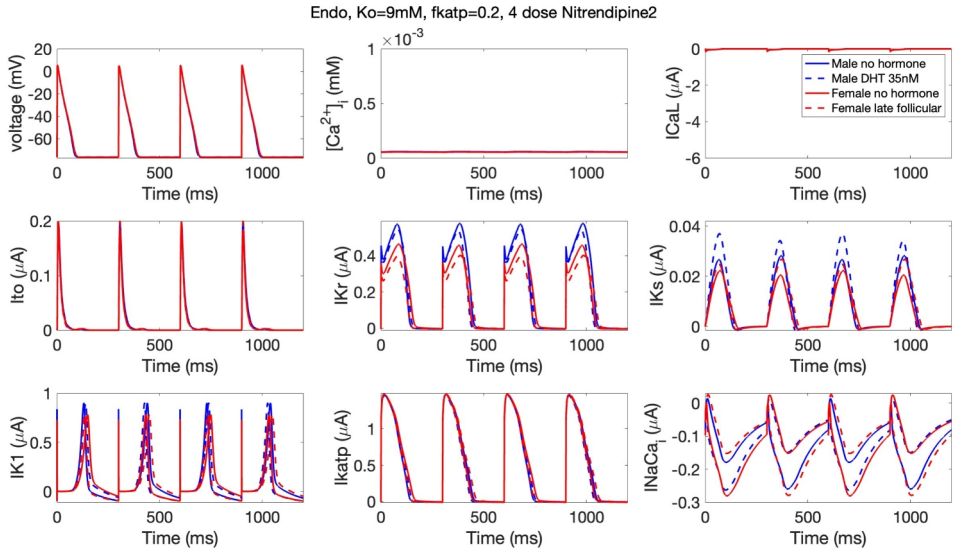


**Fig. 7**. Time-evolution profiles of voltage and ionic currents in the endocardium during myocardial ischemia under the treatment of 4 dose Nitrendipine for males without hormone (blue), males with DHT 35 nM (blue dashed), females without hormone (red), and females in the late follicular phase (red dashed), with a fixed BCL of 300 ms, where $f_{\mathrm{katp}} = 0.2$ and $[\mathrm{K}^+]_{\mathrm{o}} = 9$ mM.

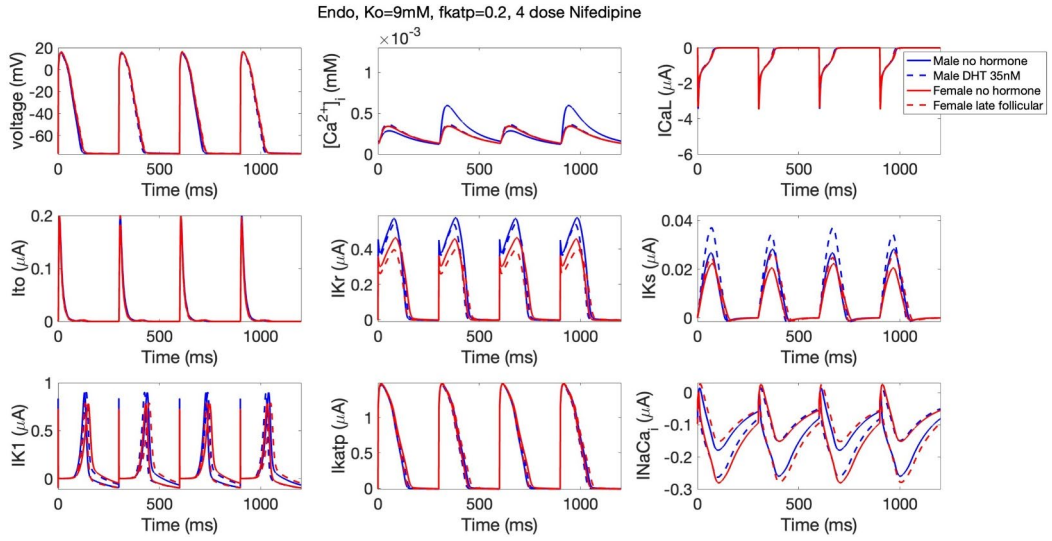


**Fig. 8**. Time-evolution profiles of voltage and ionic currents in the endocardium during myocardial ischemia under the treatment of 4 dose Nifedipine for males without hormone (blue), males with DHT 35 nM (blue dashed), females without hormone (red), and females in the late follicular phase (red dashed), with a fixed BCL of 300 ms, where $f_{\mathrm{katp}} = 0.2$ and $[\mathrm{K}^+]_{\mathrm{o}} = 9$ mM.

reperfusion. Nevertheless, it also highlights the need for further investigation into the broader implications on cardiomyocyte electrical stability and potential arrhythmogenic risks.

Understanding sex differences in myocardial ischemia is crucial due to varied clinical presentations, pathophysiological mechanisms influenced by biological and hormonal factors, and distinct treatment responses between men and women. Women often exhibit different symptoms than men[39–45], which can lead to underdiagnosis or delayed treatment[46,47]. Investigating these differences can provide insights into disease mechanisms, optimize therapeutic approaches tailored to each sex, and improve overall treatment outcomes. Additionally, identifying sex-specific risk factors informs targeted prevention strategies and enhances public health efforts to mitigate cardiovascular disease disparities. Integrating sex-specific data into research and clinical guidelines promotes personalized medicine and ensures equitable healthcare practices, ultimately advancing cardiovascular health outcomes for diverse populations.

In the context of myocardial ischemia, the phenomena of cardiac alternans and the 2:1 response are significant markers that reveal underlying electrophysiological dynamics within the heart. Cardiac alternans refers to a phenomenon where there is a beat-to-beat alternation in cardiac contractions, which can manifest as variations in the strength or duration of heartbeats. This phenomenon has emerged as a potentially critical indicator of the heart's stability and responsiveness under ischemic conditions. The presence of alternating patterns during ischemia suggests an enhanced risk of arrhythmias and other cardiovascular complications.

The interplay between cardiac alternans and myocardial ischemia is particularly pronounced, as ischemia affects the heart's electrical stability and mechanical performance. When myocardial ischemia occurs, reduced blood flow leads to decreased oxygen availability and metabolic disturbances. These changes can modify APD in cardiac myocytes, resulting in the alternans observed in electrocardiographic recordings. Studies have shown that during ischemia, the mechanisms underlying electrical alternans may include variations in calcium handling, changes in repolarization dynamics, and the influence of ischemic metabolites that affect cardiac myocyte excitability.

The implications of understanding the relationship between cardiac alternans, 2:1 responses, and myocardial ischemia are profound for both diagnosis and treatment. Cardiac alternans can serve as a predictive marker for patients at risk of arrhythmias during ischemic events, and monitoring these alternans could become a vital tool for clinicians. In particular, recognizing that females are more likely to exhibit these patterns during treatment can inform more tailored and effective therapeutic strategies, potentially involving adjustments in medication, pacing techniques, or other interventions designed to stabilize heart rhythms under ischemic stress.

Using single-cell models to investigate sex differences in myocardial ischemia poses significant limitations and challenges. These models simplify the intricate interactions involved in myocardial ischemia, potentially overlooking the broader physiological and systemic influences. They may not fully capture the spectrum of differences between males and females, including genetic variations, hormonal influences, and physiological responses. Furthermore, these models lack the contextual information from the tissue microenvironment and systemic factors such as hormonal dynamics, which are critical for comprehensively understanding sex-specific responses. Validating the insights gained from these models against clinical outcomes requires robust experimental and clinical studies, which are often constrained by the availability of comprehensive sex-specific data and ethical considerations. Bridging the gap between cellular observations and clinical applicability necessitates integrating single-cell findings with multi-scale approaches that account for systemic and environmental factors in myocardial ischemia research.

The future research aims to explore the roles of hormones and genes in these differences. Long-term studies observing how treatments tailored for men and women work in real-world settings could validate the findings. Enhancing the current modeling techniques to study these differences at the tissue level will advance personalized treatment strategies for heart diseases based on gender.

## Methods

### Model myocardial ischemia

A modified human ventricular model, based on[48] and an updated version of the O'Hara human cell model (ORd)[49], was used to investigate the electrophysiological properties under ischemic conditions. The original ORd model has certain limitations in modeling myocardial ischemia, particularly in simulating post-repolarization refractoriness (PRR) in single cells and hyperkalemia within tissue. Notably, it fails to replicate excitation propagation when the extracellular potassium concentration ($[K^+]_o$) exceeds 6 mM. To address these limitations, the modified ORd model changes the steady state of the inactivation $h$ gate in INa as described in[50] and further improves tissue propagation under hyperkalemia by modifying the time constants of the inactivation $h$ and $j$ gates of INa to match the INa formulation of the TP06 model in[51] (see[48] for details). These changes enable the modified ORd model to reproduce the observed increase in PRR under ischemic conditions in single cells and to allow excitation propagation in tissue with elevated $[K^+]_o$.

This study focused on simulating the electrophysiological effects during the initial phase of acute myocardial ischemia, typically within the first 10 to 15 min. This timeframe is crucial as it represents the period with the highest arrhythmic risk following the onset of ischemia[5,11,14–16,52–55]. To mimic hyperkalemia and hypoxia, the following modifications to the model was introduced. (1) The adenosine triphosphate sensitive potassium current $I_{\mathrm{Katp}} = G_{\mathrm{Katp}} f_{\mathrm{Katp}} \left(\frac{[K^+]_o}{[K^+]_{n,o}}\right)^{0.24} (V_m - E_K)$ was added to the ORd model, where $f_{\mathrm{Katp}}$ controls different levels of ischemia, and $[K^+]_{n,o}$ represents the normal extracellular potassium concentration, which is 5.4 mM. (2) Hyperkalemia and hypoxia are simulated by adjusting $[K^+]_o$ and $f_{\mathrm{Katp}}$ to reflect the range of values observed experimentally, spanning from control to ischemic conditions[16,17,56,57], based on the gradient between ischemic and healthy regions[13,56,58]. Specifically, $[K^+]_o$ was increased from 4 to 9 mM in 1 mM increments. $f_{\mathrm{Katp}}$ was increased from 0 to 0.2, in steps of 0.02 for single cells. Additionally, the peak conductances of INa and ICaL were decreased by 25% in all simulations.

### Model sex difference

The sex-specific ORd model was used, as described in[45,59], to simulate endocardial and epicardial cells for both genders. This model incorporates experimentally identified genomic differences and the effects of sex steroid hormones on key cardiac ion channels. Specifically, the updated version outlined in[59] was utilized, which integrates functional sex disparities in $Ca^{2+}$ handling to more accurately reflect experimental findings[60–67]. For instance, adjustments were made to the maximal transport rate of the $Na^+$-$Ca^{2+}$ exchanger (NCX) in the female model, increasing it by 15%, while previously identified differences between females and males in SERCA and $Na^+$ /$K^+$-ATPase (NKA) formulations were removed in accordance with experimental evidence[60,68].

The immediate effects of physiologically relevant concentrations of sex steroid hormones (testosterone, progesterone, and estrogen) on human physiology were examined. The male model accounted for the effects of two testosterone concentrations (10 nM and 35 nM) on the critical plateau currents, including IKr, IKs, and ICaL, by applying the appropriate scaling factors to these currents. The female model, on the other hand, incorporated the effects of estrogen and progesterone on the same currents using the corresponding scaling factors. In particular, the female model underwent adjustments with estrogen and progesterone concentrations corresponding to three menstrual cycle stages: early follicular phase (estrogen at 0.1 nM and progesterone at 2.5 nM), late follicular phase (estrogen at 1 nM and progesterone at 2.5 nM), and luteal phase (estrogen at 0.7 nM and progesterone at 40.6 nM). Further details are provided in the supplementary material of[45].

## Drug stimulation

The effects of various drugs, administered immediately after the onset of acute myocardial ischemia, were simulated using both male and female models. The medications included Bepridil, Sotalol, Diazepam, Diltiazem, Mibefradil 1, Mibefradil 2, Nifedipine 1, Nifedipine 2, Nitrendipine 1, Nitrendipine 2, Prenylamine, Propranolol, Verapamil, and Ranolazine, as discussed in[59]. These drugs were evaluated using a pore block model within the CiPA assessment framework. This included incorporating IC50 values, hill coefficients, maximal concentration (Cmax), and dose for each drug across various ion channels. For instance, the effects of drugs on IKr, ICaL, INaL, INa, Ito, IKr, IK1 and IKs were taken into account, as detailed in[45,59]. Voltage traces, $[Ca^{2+}]_i$, ICaL, Ito, IKr, IKs, IK1, IKatp, and INaCa were then extracted for further analysis.

## Numerical methods

Single cell simulations were performed in MATLAB for all models. Equations were solved using ode15s with a time step of 0.01 ms, and a relative and absolute tolerance of $10^{-7}$ and $10^{-9}$, respectively, to ensure numerical convergence. Specifically, a single-cell simulation was run over 1000 beats, using stimuli of 0.5 ms and 80 $\mu A/\mu F$ in amplitude. The APD for the last two beats out of each 1000-beat sequence was calculated. If the difference in APD between the last two beats is less than 3 ms, the system is considered to be in a steady state with a 1:1 response. If the difference exceeds 3 ms, additional pacing is required until the steady state is achieved. If 1:1 behavior is not observed, additional assess is needed for the presence of 2:2 pattern by evaluating the stability of every other beat. In particular, the APDs for every other beat over the last four beats out of every 1000 beats were calculated. If the difference in APD between each pair of beats is less than 3 ms, the system is considered to be in a steady state of 2:2 response. If 1000 beats are not sufficient, pacing continues until the steady state is reached.

A BCL of 300 ms was applied during periodic pacing to simulate the rapid heart rate observed under ischemic conditions. In the single-cell simulation, the APDs for the last four beats were calculated, defined as the duration from depolarization (where the derivative of membrane potential with respect to time, $\frac{dV}{dt}$, is greater than 0) to repolarization, which occurs when the membrane potential falls below $-60$mV.

## Data availability

All the data is provided within the manuscript. The code for this paper is available on GitHub: https://github.com/nweipd/Sex-specific-disparities-in-electrophysiological-responses-and-drug-reactions-

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## Author contributions

Ning Wei designed the numerical experiments, conducted the simulations, wrote the manuscript, and edited the final version.

## Declarations

## Competing interests

The authors declare no competing interests.

## Additional information

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