**Methods** 18 healthy male hybrid dogs aged 15—18 months were divided into three groups randomly, the control group (n=6), the Perindopril group (1 mg/kg/d, n=6), the Spironolactone group (10 mg/kg/d, n=6). To test the form and function of left atrial and plasma aldosterone levels before pacing and 4 weeks, 8 weeks after pacing, respectively. Observe the number of dogs maintained AF and duration of AF after cessation of pacing. Then, kill the animals and collect some tissues of left and right atrial to detect aldosterone levels and test the situation of atrial fibrosis by pathological examination. By comparing the similarities and differences between the three groups, to understand the impact of atrial interstitial remodelling and the occurrence and development of atrial fibrillation induced by aldosterone inhibitor.

**Results** The levels of plasma aldosterone were no significant differences between the three groups before pacing ($p>0.05$), while 4 weeks and 8 weeks after pacing the plasma aldosterone levels and the aldosterone levels of atrial tissue 8 weeks after pacing of the other groups were significantly lower than those of the control group ($p<0.05$). In the control group, 4 weeks and 8 weeks after pacing the plasma aldosterone levels was significantly higher than that before pacing ($p<0.05$), while in the other two groups, there were no significant differences between before and after pacing ($p>0.05$). Pacing for 4 weeks and 8 weeks later, the diameter, end-systolic volume and end-diastolic volume of the left atrium of the control group dogs significantly increased than before pacing, and left atrial ejection fraction (LAEF) lower than before pacing significantly ($p<0.05$). Compared with the control group, those of the Perindopril group and Spironolactone group after pacing significantly reduced, but LAEF significantly increased ($p<0.05$). Compared with the control group, the number of dogs maintained atrial fibrillation of the two treatment groups after cessation of pacing significantly reduced, with a shorter average duration of atrial fibrillation. While there was not significant difference between the two treatment groups. The value of Collagen Volume Fraction (CVF) of the Control group was significantly higher than those of the other two groups ($p<0.05$), while no significant difference value between the two treatment groups ($p>0.05$).

**Conclusion** The aldosterone receptor antagonist (spironolactone) and ACEI (Perindopril) can inhibit aldosterone levels and atrial fibrosis, improve the changes of atrial structure and function, and reduce the incidence and duration of atrial fibrillation. And the effects of the two drugs are similar.

## e0097 CANINE MARROW MESENCHYMAL STEM CELLS WITH LENTIVIRAL MHCN4 GENE TRANSFER CREATE CARDIAC PACEMAKERS

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The purpose Research on biological pacemakers for the heart has so far mainly focused on short-term gene and cell therapies. To develop a clinically relevant biological pacemaker, long-term function is crucial. Lentiviral vectors can mediate long-term gene expression. The purpose of the present study was to determine whether canine mesenchymal stem cells (cMSCs) provide impulse initiation over 2 weeks without the use of immunosuppression.

**Methods** pacemaker current (If) were studied in cMSCs that overexpressed isoform 4 of the Hyperpolarization-activated Cyclic Nucleotide-gated channel (encoded by HCN4) after lentiviral gene transduction. HCN4 protein expression was confirmed by cellular immunofluorescence staining and western blotting. $3\times10^6$ cMSCs transfected with either control plasmid (EGFP) or HCN4 gene construct were injected subepicardially in the canine right ventricular wall in situ.

**Results** Perforated patch-clamp experiments demonstrated that HCN4- transduced single canine mesenchymal stem cells exhibited a fivefold higher if than non-transduced single cMSCs, expressed high levels of Cs-sensitive current, confirming the expressed current as If-like. After cMSCs injected 2 weeks, during sinus arrest, all control (EGFP) hearts had spontaneous atrioventricular node rhythms; In the EGFP-mHCN4 group, 4 of 6 animals developed spontaneous ventricular rhythms; 2 of 6 animals developed spontaneous atrioventricular node rhythms. Moreover, immunostaining of the injected regions demonstrated the presence of cMSCs forming gap junctions with adjacent myocytes and manifested no cellular or humoural rejection at that time.

**Conclusion** These studies demonstrate that genetically modified cMSCs can express functional HCN4 channels in vitro and in vivo, mimicking overexpression of HCN4 genes in cardiac myocytes, and represent a novel delivery system for pacemaker genes into the heart.

## e0098 HIF-1α, SDF-1α AND VEGF GENE EXPRESSION AFFECTED BY HIF-1α SIRNA IN MSCS

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**Objective** HIF-1a, SDF-1a and VEGF gene expression affected by HIF-1a siRNA in MSCs.

**Methods** Bone marrow mesenchymal stem cells were cultured in vitro (3—5 generation,), inverted microscope was used to observed morphology, flow cytometry was used to detective of surface markers CD11b /c, CD34, CD44 and CD90. MSCs were divided into five groups, hypoxia group (only hypoxia), liposome group (MSCs transfected with empty liposomes), siRNA609 (MSCs transfected with siRNA609 sequence), siRNA658 (MSCs transfected with siRNA658 sequence), siRNA2070 (MSCs transfected with siRNA2070 sequence). The cells were cultured under hypoxia for 24 h, HIF-1a gene expression was dectived by RT-PCR. MSCs were divided into four groups, control group (without any treatment), hypoxia group (hypoxia 24 h), liposome control group (MSCs transfected with liposome then hypoxic 24 h), RNA interference (MSCs transfected with RNA interference sequences then hypoxic 24 h). MSCs mRNA expression RT-PCR, MSCs supernatant protein levels was detected by ELISA, MSCs hypoxic supernatant stimulate rat smooth muscle cell proliferation was detected by CCK8.

**Results** 1. Flow cytometry detective CD11b /c negative, CD34 negative, CD44 positive, CD90 positive cells respective reached 84.2%±0.2%, 97.91%±0.7%, 99.8%±0.9%, 97.7%±0.4% and CD44+/CD34—cell number reached 99.4%±0.4%. 2. SiRNA transfected cells could be detected the green fluorescent signal, lipsome group and the control group could not be detected the green fluorescence signal. 3. RT-PCR results showed that siRNA609, siRNA658, siRNA2070 group compared with hypoxia group HIF-1a gene expression was lower ($p<0.05$), the siRNA658 group is the least ($p<0.05$). 4. RT-PCR results revealed that compared with the normal control group hypoxia group HIF-1 a, SDF-1 a and VEGF gene expression increased ($p<0.05$), and compared with the lipsome control group RNA interference group HIF-1 a, SDF-1 a and VEGF gene expression reduced ($p<0.05$). 5. ELISA results revealed that compared with the normal control group hypoxia group HIF-1 a, SDF-1 a and VEGF content were increased ($p<0.05$), compared with the liposome control group RNA interference group HIF-1 a, SDF-1 a, VEGF content was decreased ($p<0.05$). 6. CCK8 results revealed that compared with the normal group control group hypoxia can promote smooth muscle cell proliferation ($p<0.05$), compared with