

The Effects of Exposure to Mild Hyperbaric Oxygen on Specific Physiological Indexes in the In Vitro Fertilization Therapy of Infertile Women

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Currently, few studies have been conducted on the effects of physiological factors during exposure to mild hyperbaric oxygen (MHBO) environment on infertile. Thirteen healthy women and seven infertile women undergoing in vitro fertilization (IVF) were exposed to MHBO environment using an oxygen room. The specific physiological data collected during the study were analyzed. Exposure to MHBO increases blood oxygen saturation and may enhance metabolism, potentially improving the chances of pregnancy in infertile women by improving the outcome of implantation. Heart rate variability (HRV) analysis showed that women in the infertile group exhibited slightly less parasympathetic nerve activity than those in the healthy group after exposure to MHBO. These findings suggest that the blood supply to the reproductive organs in infertile women may not yet be sufficient. We hypothesized that further enhancement of blood flow through exposure could improve implantation success in women with infertile. Furthermore, decreased parasympathetic activity in infertile women may be one of the causes of infertile, as increased sympathetic activity prevents mental relaxation. This study is a key step in investigating the mechanisms underlying infertile. *Shinshu Med J* 73 : 275—284, 2025

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Key words : mild hyperbaric oxygen, infertile, heart rate variability, metabolism, poincaré plot

Abbreviations : MHBO, Mild Hyperbaric Oxygen ; IVF, in vitro fertilization ; HRV, Heart Rate Variability ; RMSSD, Root Mean Square of Successive R-R interval Differences ; HF, High Frequency Spectra ; LF, Low Frequency Spectra ; SD1, Poincaré Plot Standard Deviation Perpendicular the Line of Identity ; SD2, Poincaré Plot Standard Deviation Along the Line of Identity.

I Introduction

The number of women with infertile in Japan is increasing. The primary causes of infertile are thought to be lifestyle changes, which may lead to reduced metabolism in reproductive organs. We hypothesized that an increase in the metabolism of the uterus and ovaries may improve the pregnancy rates and that exposure to mild hyperbaric oxygen

(MHBO) may help increase of metabolism. A preliminary study by Yoshikawa et al.¹⁾ conducted in Suwa Maternity Clinic using an oxygen capsule found that exposure to MHBO improved pregnancy rates, and increased birth among infertile women. However, owing to the limited space within the oxygen capsule, the researchers were unable to measure specific physiological data to explore the underlying mechanism of these effects. Therefore, in this study, we examined the effects of exposure to MHBO on specific physiological indices in both healthy women and infertile women who were undergoing IVF therapy. To replicate the conditions of the previous studies

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Oxygen Room

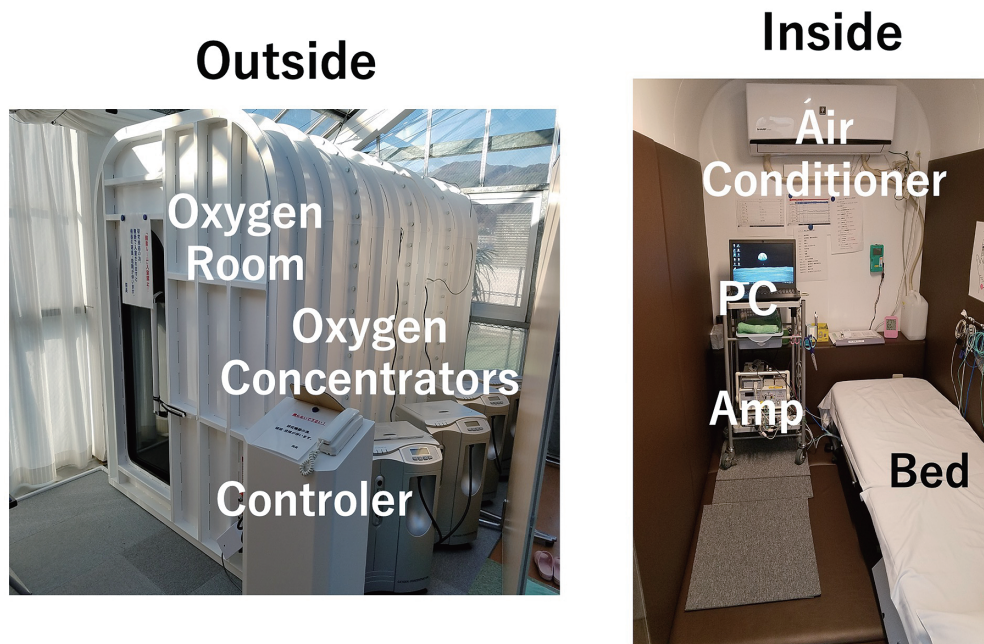


Fig. 1 Left picture shows outside of the oxygen room, a controller of air pressure and oxygen concentrators.

Right picture shows inside of the oxygen room, PC, amplifiers and a bed for the subject.

while allowing for more comprehensive data collection, we used an oxygen room instead of an oxygen capsule. Our findings will provide insights into the mechanism by which MHBO improves implantation in infertile women.

II Materials and Method

Thirteen healthy normal women (34.2 ± 6.1 years old) and 7 infertile women (37.0 ± 1.9 years old) who had undergone three embryo transfers without implantation during IVF therapy in Suwa Maternity Clinic participated in this study, and the physiological responses of the participants to MHBO were investigated. Measurements were carried out continuously, and the data were stored on a PC for further analysis using the Power Lab System (AD Instruments, USA).

An oxygen room (Nihon Kiatsu Balk Kogyo, Japan ; width : 1,500 mm, height : 1,900 mm, depth : 2,560 mm) was used in this study (**Fig. 1**). The system consists of three instruments: the main part is an oxygen room made of iron, measuring amplifiers, a bed, and

an air conditioner inside the room. Air pump controller and three oxygen concentrators are equipped outside the oxygen room.

Physiological data were measured at just before loading of MHBO, during study and at 15min after released MHBO.

The measured parameters included the following : continuous recording of ECG (AD Instruments/ Power Lab System, USA), percentage of blood oxygen saturation/ SaO_2 (Fukuda Electric Corporation/ ML320-F, Japan), skin temperature (Fukuda Electric Corporation/SST-1, Japan), skin blood flow on the finger and an earlobe measured by a laser flow meter (Advance/ALF21D, USA), heart rate and RR-interval (AD Instruments/Power Lab System, USA), oxygen concentration (JIKCO/JKO-25, Japan), and barometric pressure (SMC World/digital pressure switch ISE30A, Japan) inside the room. Additionally, blood pressure was measured before and after the study using a manometer (Fukuda Electric Corporation/FB-270, Japan). Heart rate variability (HRV) analysis²⁾ was performed using the stored data to

Raw data of healthy woman

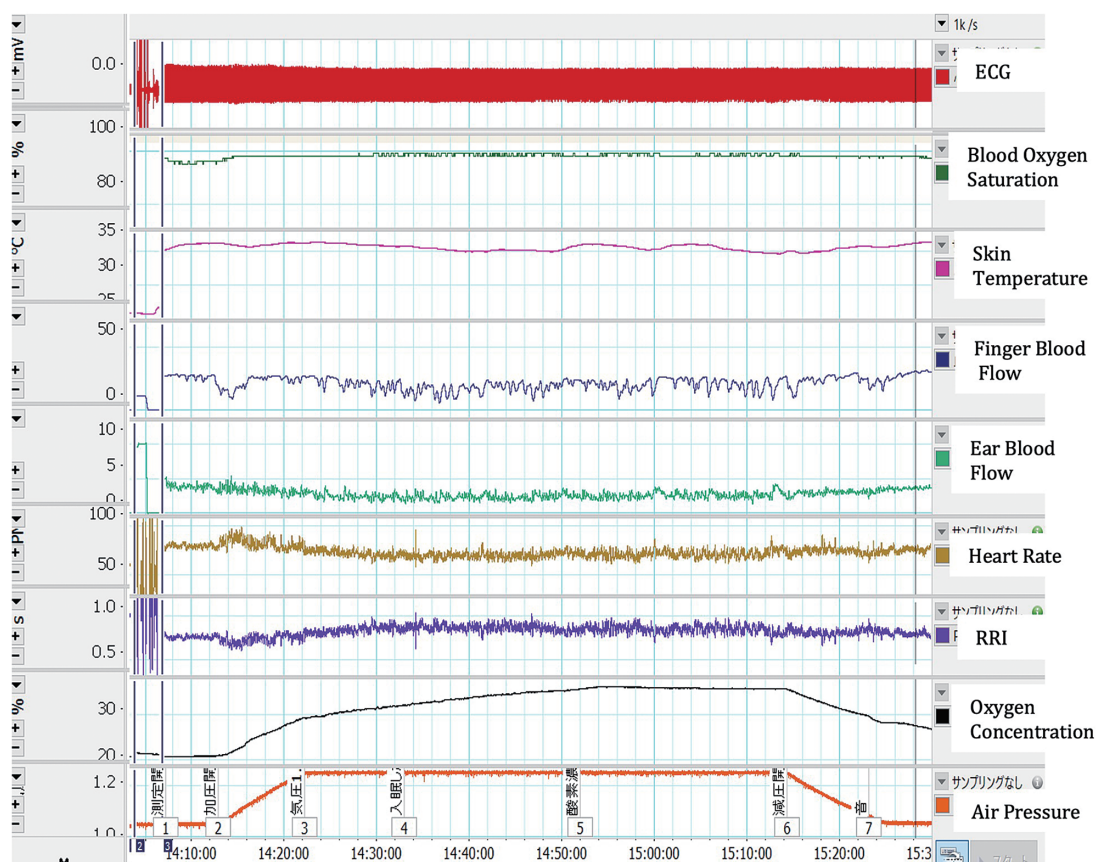


Fig. 2 This figure shows one case of the raw data on specific physiological indexes of healthy woman during exposure to mild hyperbaric oxygen.

Physiological indexes are ECG, blood oxygen saturation, skin temperature, finger skin blood flow, ear robe skin blood flow, heart rate, RR-interval, oxygen concentration in the room, and air pressure in the room.

assess autonomic nerve activity via the Power Lab System.

This study was non-invasive, and ethical approval was granted by the Suwa Maternity Clinic Committee of Ethics in Human Research (No.2022-01). It was also registered with UMIN (University Hospital Medical Information Network/UMIN000044498). All participants provided written informed consent. Sensors for each measurement parameter were attached to the participant, and the study was conducted in the supine position in a quiet and dark environment. Exposure to MHBO lasted 60 min, and the barometric pressure in the room was maintained at 1.25 hPa. The temperature in the room was maintained at $22 \pm 3^\circ\text{C}$ and humidity was maintained at

$40 \pm 10\%$. A female staff member was in the oxygen room observing the participant and adjusting the equipment during the test. Other staff member was outside the room adjusting other equipment and ensuring the security of the test.

Means and standard deviations were calculated using the standard procedures. Student's t-test was used for statistical comparisons, with significance indicated by P-value $^* < 0.05$ and $^{**} < 0.01$.

III Results

A Effects of exposure to MHBO on physiological indexes and on HRV in healthy women group

Fig. 2 presents an example of the raw data for each physiological parameter recorded during the

Table 1 Results of exposure to MHBO

		SaO ₂ (%)	Temp of finger skin (°C)	Blood flow of finger skin (ml/min/100g)	Blood flow of ear skin (ml/min/100g)	Heart rate (bpm)	RR interval (sec)	SBP (mmHg)	DBP (mmHg)
Healthy women n = 103	Before loading	95.6 ± 1.1	35.6 ± 1.6	31.1 ± 12.6	6.4 ± 3.4	73.1 ± 11.9	0.84 ± 0.1	110.8 ± 9.5	67.3 ± 7.5
	After loading	98.2** ± 0.5	35.3 ± 1.7	27.4* ± 12.0	6.0* ± 4.0	68.0** ± 12.1	0.91** ± 0.2	105.1* ± 7.8	66.0 ± 7.2
	Percentage of changes (%)	3.0** ± 1.0	3.0 ± 12.4	0.7 ± 144.9	-1.3* ± 52.1	-7.3** ± 8.3	9.3** ± 11.4	-5.3** ± 7.0	-2.1* ± 8.0
Infertile women n = 68	Before loading	95.5 ± 1.2	33.5 ± 4.2	28.6 ± 16.6	14.3 ± 11.8	66.0 ± 9.3	0.92 ± 0.1	108.4 ± 12.8	72.3 ± 14.1
	After loading	98.2** ± 0.7	34.4* ± 2.2	22.3** ± 12.2	8.9** ± 6.7	63.1** ± 9.0	0.97** ± 0.1	105.4** ± 9.9	70.7** ± 11.2
	Percentage of changes (%)	2.7* ± 1.2	-1.2* ± 7.1	-3.0 ± 76.9	-27.4** ± 48.1	0.5** ± 0.1	4.7** ± 8.8	0.5** ± 0.1	1.6 ± 10.5
Significance of percentage of changes between Healthy women and Infertile women (P value)		>0.05	0.01	>0.05	0.01	<0.01	0.02	<0.01	>0.05

This table shows the results of exposure to mild hyperbaric oxygen.

In this table, percentage of changes of parameters between before and after loading mild hyperbaric oxygen were showed.

Upper part shows the results of specific physiological indexes in healthy women (n = 103 trials of 13 women). Values of systolic blood pressure and diastolic blood pressure are added.

Lower part shows the results of specific physiological indexes in infertility women (n = 68 trials of 7 women)

Values are presented as the mean ± standard deviation

Significant differences of the after loading compared to the before loading were shown as * < 0.05 and ** < 0.01

study. **Table 1** summarizes the changes in all parameters before and after exposure to MHBO in both the healthy women group and the infertile women group.

During exposure to MHBO, the air pressure in the room increased by 1.25 hPa within approximately 10 min. The oxygen concentration in the room gradually increased and reached 36 ± 1 % approximately 40 min after exposure to MHBO and maintained at this level for approximately 20 min. Oxygen saturation increased and was maintained at the level observed immediately after exposure to MHBO. In the **Table 1**, except the temperature of finger skin and the diastolic blood pressure, other parameters showed significant ($P < 0.01$ or $P < 0.05$) changes after loading of MHBO. And percentage of changes except temperature of finger skin and blood flow of finger skin showed significant changes ($P < 0.01$ or $P < 0.05$) after loading.

In HRV analysis, we assessed the autonomic nerve activity before and after exposure to MHBO. The

Poincaré plot was first used to assess autonomic nerve functions. **Fig. 3** illustrates changes in the Poincaré plot before and after exposure to MHBO. The distribution of beat-to-beat variability in the Poincaré plot shifted to the upper right in the histogram of the R-R interval of the ECG. Other HRV parameters are presented in the upper part of **Table 2**. Notably, the average RMSSD increased significantly ($P < 0.01$) after exposure to the MHBO compared to before loading. And percentage of changes of parameters between before and after loading presented also in the **Table 2**. These parameters of percentage of changes except SD2/SD1 in HRV before and after loading showed significant ($P < 0.01$ or $P < 0.05$) changes.

B Effects of exposure to MHBO on physiological indexes and on HRV in infertile women group

An example of the raw data on exposure to MHBO in infertile woman is shown in **Fig. 4**. The responses to each physiological item were similar to those

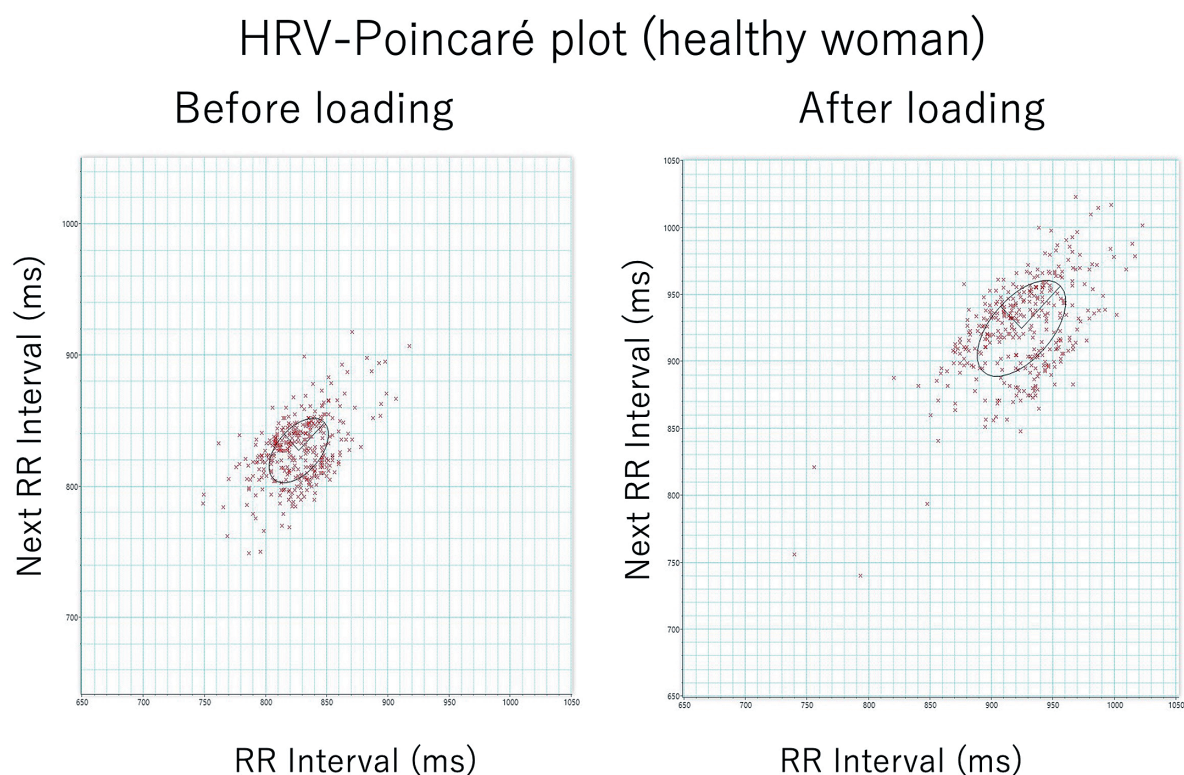


Fig. 3 This figure shows one case of the change in the distribution of HRV-Poincaré plot before and after loading of mild hyperbaric oxygen in healthy woman.

Table 2 Results of exposure to MHBO (HRV)

Healthy women														
	Before Loading							After Loading						
	LF (ms ²)	HF (ms ²)	LF/HF (%)	RMSSD (ms)	SD1 (ms)	SD2 (ms)	SD2/SD1	LF (ms ²)	HF (ms ²)	LF/HF (%)	RMSSD (ms)	SD1 (ms)	SD2 (ms)	SD2/SD1
Average	390.0	655.8	0.924	30.3	22.3	47.1	2.55	724.8**	832.0	1.421**	40.9**	28.9**	61.9**	2.64*
SD	784.5	1859.3	0.839	18.8	16.1	20.3	1.11	782.9	1056.9	1280	24.2	16.9	22.3	1.35
Percentage of changes between Before and After Loading (%)								281.1* ± 365.1	122.2* ± 150.3	90.6* ± 153.5	45.0** ± 61.9	45.1* ± 61.8	42.7** ± 48.2	8.2 ± 45.2
Infertile women														
	Before Loading							After Loading						
	LF (ms ²)	HF (ms ²)	LF/HF (%)	RMSSD (ms)	SD1 (ms)	SD2 (ms)	SD2/SD1	LF (ms ²)	HF (ms ²)	LF/HF (%)	RMSSD (ms)	SD1 (ms)	SD2 (ms)	SD2/SD1
Average	1091.5	1312.9	1.048	51	35.6	77.4	2.35	1745.7**	1570.3*	1.399**	54.8*	38.7*	95.2**	2.67**
SD	1116.4	1238.5	0.859	27.1	18.5	35.0	0.71	1889.2	1426.1	1.002	28.0	21.0	48.9	0.87
Percentage of changes between Before and After Loading (%)								93.5 ± 147.1	43.3 ± 115.2	5.4 ± 85.8	11.6* ± 43.3	12.7* ± 43.2	22.5* ± 28.3	17.5 ± 37.5
Significance of percentage of changes between Healthy women and Infertile women (P value)								LF (ms ²)	HF (ms ²)	LF/HF (%)	RMSSD (ms)	SD1 (ms)	SD2 (ms)	SD2/SD1
								<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	>0.05

This table shows the results of HRV analysis before and after loading of mild hyperbaric oxygen.

In this table, percentage of changes of parameters between before and after loading were showed.

Upper parts show the results in healthy women (n=103 trials of 13 women) and lower parts shows the results in infertility women (n=68 trials of 7 women).

Values are presented as the mean ± standard deviation

Significant differences of the after loading compared to the before loading were shown as * < 0.05 and ** < 0.01

Raw data of infertile woman

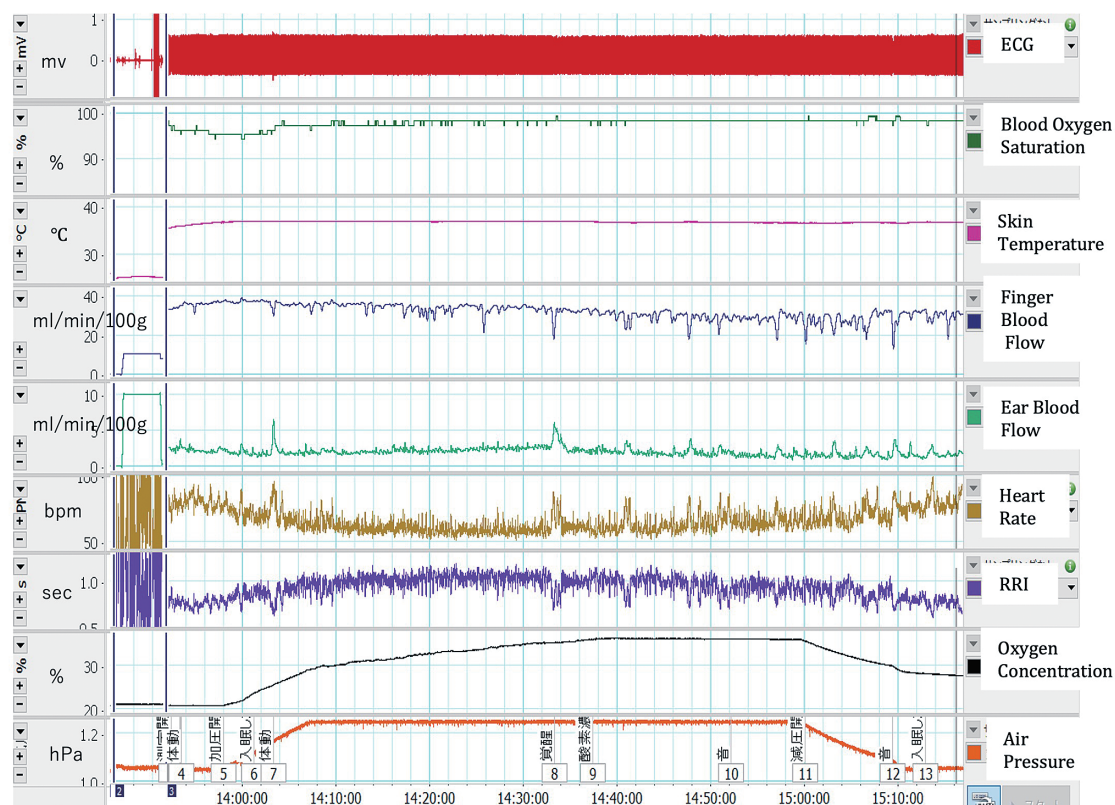


Fig. 4 This figure shows one case of the raw data on specific physiological indexes of infertile woman during exposure to mild hyperbaric oxygen.

Physiological indexes are ECG, blood oxygen saturation, skin temperature, finger skin blood flow, ear robe skin blood flow, heart rate, RR-interval, oxygen concentration in the room, and air pressure in the room.

observed in healthy women. However, the heart rate decreased significantly compared with that in healthy women. The time-course changes in all the parameters are shown in the middle of **Table 1**. All parameters after exposure to MHBO increased significantly ($P < 0.01$ or $P < 0.05$) compared to the baseline average. And the percentage of changes except blood flow of finger skin and diastolic blood pressure showed significant ($P < 0.01$ or $P < 0.05$) changes before and after loading of MHBO.

Fig. 5 shows the change in the Poincaré plot before and after loading of MHBO. The Poincaré plot demonstrating a pattern similar to the change observed in those in the healthy women group. In this case, the distribution of heartbeats in the Poincaré plot shifted toward the upper right of the histogram of the R-R interval after loading. Additionally, HRV

parameters are presented in the middle of **Table 2**. Moreover, significant increases were observed in the all parameters after loading compared to the before loading value. Percentage of changes of these parameters showed in the table. And only RMSSD, SD1 and SD2 showed significant ($P < 0.05$) change.

C Comparison physiological response to MHBO between healthy women and infertile women

Table 1 presents a comparison of the data obtained before and after loading of MHBO between healthy women and infertile women. The changes in most parameters upon exposure to MHBO were similar in both groups. Blood oxygen saturation increased significantly after loading in both groups. Significance of percentage of changes between two groups showed in the bottom of **Table 1**. Only SaO_2 , blood flow of finger skin and diastolic blood pressure had no

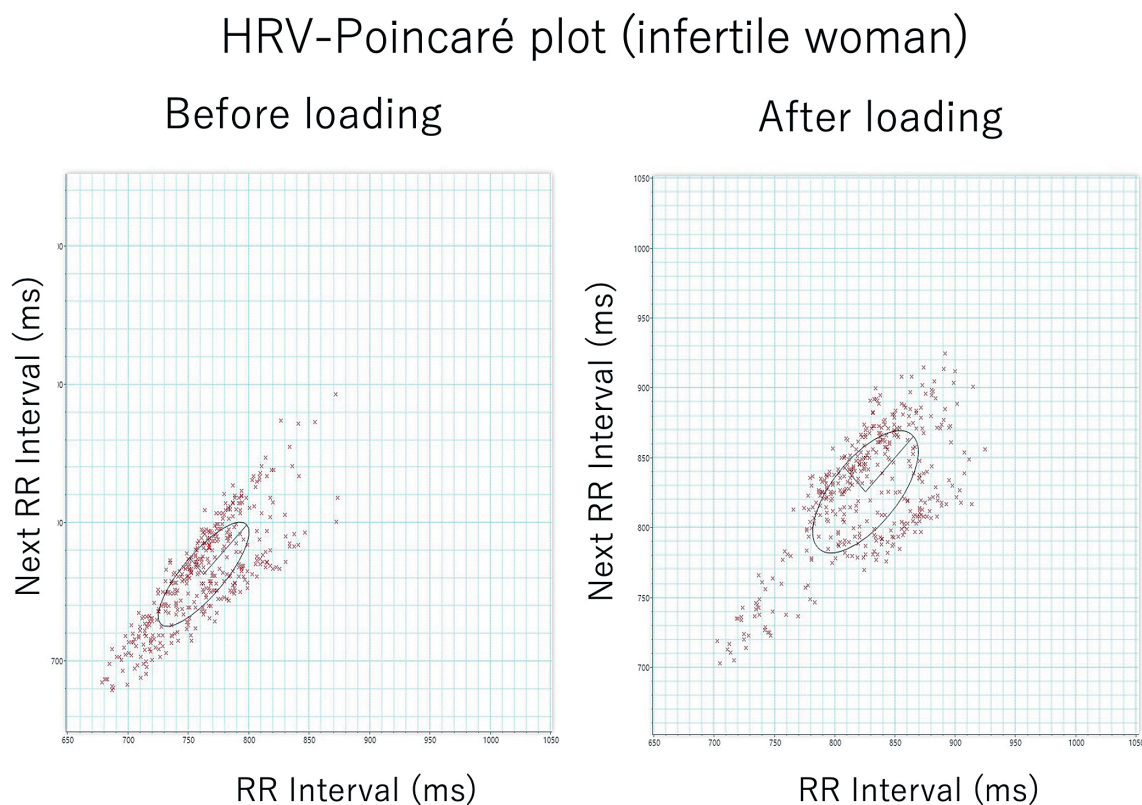


Fig. 5 This figure shows one case of the change in the distribution of HRV-Poincaré plot before and after loading of mild hyperbaric oxygen in infertile woman.

significant changes between two groups. However, percentage of changes of most parameters in healthy women were larger than infertile women after loading.

Overall, the changes in the HRV index were similar in both groups after loading of MHBO in **Table 2**.

Significance of most percentage of changes between two groups showed high P-values except SD2/SD1. However, percentage of changes between before and after loading, healthy women showed larger value than infertile women in most parameters. These results may show that MHBO were more effective in healthy women than in infertile women. In addition, SD1 and SD2 showed larger values in healthy women and the points were vary in the Poincaré plot distribution, and this indicates that the increase in parasympathetic nerve activity is more remarkable in healthy women than in infertile women.

IV Discussion

A MHBO exposure and the assessment of the autonomic activity

Yoshikawa et al.¹⁾ reported the beneficial effects of MHBO exposure in infertile women. In this study, we used an oxygen room instead of an oxygen capsule at 1.25 hPa with 36 ± 1 % oxygen in both healthy women and infertile women. Specific physiological data were collected from the participants during the study. Prior to the main experiment, a preliminary study was conducted to confirm the safety of MHBO exposure in healthy women using an oxygen room. Notably, no evidence of oxidative stress or other adverse effects were observed in any of the participants.

To assess autonomic activity, we used HF, LF, LF/HF, RMSSD, SD1, SD2, SD2/SD1, and the distribution of Poincaré plots in the HRV analysis. In particular, RMSSD is known to reflect parasympathetic activity²⁾. On the other hand, in the frequency domain, HF also reflects parasympathetic activity, and LF reflects sympathetic activity; however, HF is influenced by respiration. Therefore, controlling respiration and maintaining stability are ideal for analyzing the fre-

quency domain of HRV. However, respiration was not controlled because of the difficulty faced by the participants in this study. As a result, we considered the influence of respiration on the frequency domain and primarily assessed autonomic nerve activity using time-domain indices, such as the distribution of the Poincaré plot, changes of RMSSD, SD1 and SD2.

B Effects of exposure to MHBO in healthy women group and infertile women group

Previous clinical human study³⁾ and experimental animal study⁴⁾ demonstrated that exposure to MHBO enhances and improves metabolism in cells and tissues. For example, Yoshikawa et al. reported the effects of MHBO exposure in infertile women¹⁾. In that study, 7 out of 37 women with intractable infertile successfully gave birth following exposure to MHBO. These findings provide important evidence supporting the role of MHBO in IVF therapy.

In this study under invasive condition, we collected specific physiological data from participants to analyze and understand the mechanisms underlying the effects of exposure to MHBO on infertile treatment, as well as the important factors contributing to these effects. Specifically, we examined physiological phenomena associated with exposure to MHBO during IVF therapy for infertility.

Under these conditions, exposure to MHBO resulted in increased blood oxygen saturation in both healthy and infertile women, a slight increase in skin temperature, a significant decrease in skin blood flow, and a significant reduction in systolic and diastolic blood pressure. In animal experiments, exposure to MHBO has been reported to reduce blood pressure in spontaneously hypertensive rats by inhibiting excessive activity of the sympathetic nerves⁵⁾. Similarly, in both groups of this study, an increase in oxygen saturation induced an increase in parasympathetic activity and a decrease in sympathetic activity, leading to a decrease in blood pressure. Another study⁶⁾ reported lower heart rates and higher peripheral oxygen saturation following exposure to MHBO, which is consistent with the results of the present study. In contrast, the skin blood flow decreased significantly in both groups. This reduction

in skin blood flow may be attributed to vasoconstriction induced by increased atmospheric pressure in the oxygen room and the elevation of blood oxygen saturation⁷⁾. However, exposure to MHBO increases blood flow in the capillaries of peripheral tissues by regulating parasympathetic and sympathetic nerve activities, thereby increasing oxygen delivery by oxygen bound to hemoglobin in red blood cells, and especially by dissolved oxygen content in plasma⁸⁾.

The assessment of HRV is inherently complex. As previously discussed, HF reflects the change in parasympathetic nerve activity, whereas LF reflects the change in sympathetic nerve activity. The LF/HF ratio correlates closely with the SD2/SD1 ratio and indicates the balance between sympathetic and parasympathetic nerves in the frequency domain analysis²⁾⁹⁾. In contrast, Poincaré plot analysis is a geometric and nonlinear method for assessing HRV dynamics⁸⁾¹⁰⁾. Poincaré plots are qualitatively evaluated using visual patterns of sympathetic and parasympathetic nerve activity⁹⁾. The interpretation of HRV analysis, particularly in the frequency domain, remains complex. Therefore, in this study, we evaluated the distribution of Poincaré plots for autonomic nerve function in addition to the change of values of RMSSD, SD1 and SD2 analysis. Poincaré plots provide a graphical representation of overall HRV and beat-to-beat variabilities¹⁰⁾, effectively visualizing the distribution of R-R interval on ECG recordings.

In infertile women, the distribution of the Poincaré plot was initially located in the lower left of the histogram of the R-R interval before exposure to MHBO. And after exposure to MHBO, a clear shift toward the upper-right quadrant was observed. Statistical results in the change values of SD1 and SD2 showed a large effect in healthy women and the points were vary in the Poincaré plot distribution. These results could indicate that the increase in parasympathetic nerve activity is more remarkable in healthy women than in infertile women. This finding suggests that infertile women, who are usually more sympathetic dominant and under greater stress compared to healthy women, and also may experience a shift toward parasympathetic dominance after exposure

to MHBO. However, in this study, statistical results indicated that the efficacy of exposure to MHBO was smaller in infertile women than that observed in healthy women. This also suggests that the parasympathetic activity may not be sufficient to improve implantation in this case.

In contrast, RMSSD is widely used to evaluate parasympathetic nerve activity²⁾¹¹⁾, and in this study, RMSSD significantly increased following exposure to MHBO. Therefore, parasympathetic nerve activity becomes predominant over sympathetic nerve activity upon exposure to MHBO, and it is possible that the internal organs may have sufficient oxygen in both groups and more blood flow in the internal organs, including the reproductive organs, than in the skin. Reports on IVF have shown that endometrial blood flow is a key determinant of uterine implantation and correlates positively with the number of viable embryos and the success of pregnancy by IVF therapy¹²⁾.

Furthermore, the heart rate significantly decreased in both groups following exposure to MHBO, indicating a relaxation response associated with increased parasympathetic nerve activity. Actually, most women in both groups had good sleep during study. Therefore, it is believed that the sympathetic nervous tension caused by external stimuli during the test is reduced. Stress is a well-documented contributor to infertility¹³⁾ as it can disrupt ovulation and impair ovarian and uterine function. In addition, excessive oxidative stress can lead to pregnancy failure¹³⁾¹⁴⁾. Therefore, exposure to MHBO may have a beneficial effect on reproductive organ disorders by adequately evading stress, optimizing cellular function, and improving their metabolism, thereby enhancing the effects of implantation.

Exposure to hyperbaric oxygen has been reported to enhance fertility in infertile women who have undergone IVF therapy, suggesting its potential as an adjunctive treatment for IVF therapy¹⁵⁾. In addition, one study reports that hyperbaric oxygen therapy may serve as an optimal intervention for improving endometrial receptivity through enhanced vascularization and oxygenation¹⁶⁾. However, these studies were conducted under high oxygen concen-

trations, which differ significantly from the conditions used in our study. Despite this difference, we hypothesize that the administration of pressurized oxygen is effective in treating infertile women. Accordingly, we investigated the effect of MHBO and proposed that exposure to MHBO may offer beneficial effects as a sub-therapy for women undergoing IVF therapy.

However, this study has a limitation due to the need for invasive methods, and to minimize the burden for the patients to avoid destruction for the treatment of infertile. Then, taking the physiological data of participants and the explanation of the data cannot be enough. Therefore, we could not show a clear basis on such as the blood flow in the reproductive organs and the metabolism in cells and tissues. In contrast, Yoshikawa et al. reported the positive effects of using an oxygen capsule to treat women with infertility¹⁾. Building on their findings, we aim to further analyze the impact of MHBO in women undergoing IVF therapy. Specifically, we will compare the differences between infertile women and pregnant women exposed to MHBO. We believe that the findings will contribute to advances in infertile treatment.

V Conclusion

Exposure to MHBO led to similar physiological changes in both healthy women and infertile women undergoing IVF therapy, including an increase in blood oxygen saturation. Both groups showed a significant increase in parasympathetic nerve activity in the HRV analysis, indicating increased peripheral blood flow and relaxation following exposure to MHBO. However, the infertile women exhibited slightly weaker parasympathetic nerve activity than the healthy women, which we believe is insufficient to improve implantation. Further analysis is needed to better understand the effects of MHBO exposure in infertile women undergoing IVF therapy.

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