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Correlation between tongue and pulse indicators and the outcome of live birth in frozen-thawed embryo transfer

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ABSTRACT

Objective To investigate the correlation between tongue and pulse indicators and the outcome of live birth in patients undergoing frozen-thawed embryo transfer (FET), as well as the association between these indicators and patients' endocrine parameters.

Methods This study was conducted at Reproductive Medicine Center, Shuguang Hospital Affiliated to Shanghai University of Traditional Chinese Medicine, Shanghai, China, from March 8, 2021 to January 5, 2022. Patients undergoing FET were divided into live birth and non-live birth groups according to their live birth outcome. The differences between the endocrine parameters [basic follicle stimulating hormone (b FSH), basic luteinizing hormone (b LH), basic estradiol (b E_2), basic progesterone (b P), basal endometrial thickness, follicle stimulating hormone (FSH) on endometrial transition day, luteinizing hormone (LH) on endometrial transition day, estradiol (E_2) on endometrial transition day, progesterone (P) on endometrial transition day, and endometrial thickness on endometrial transition day] and the tongue and pulse indicators [tongue body (TB)-L, TB-a, TB-b, tongue coating (TC)-L, TC-a, TC-b, perAll, perPart, h_1, h_4, h_5, t_1, h_1/t_1, and h_4/h_1] of patients in the two groups were analyzed, with the correlation between these variables analyzed as well using Spearman's correlation coefficient. Multivariate logistic regression was employed to identify the influential factors in the live birth prediction models across various datasets, including Model 1 consisting of endocrine indicators only, Model 2 solely consisting of tongue and pulse indicators, and Model 3 consisting of both tongue, pulse, and endocrine indicators, as well as to evaluate efficacy of the models derived from different datasets.

Results This study included 78 patients in live birth group and 144 patients in non-live birth group. Compared with non-live birth group, live birth group exhibited higher levels of TB-L (P = 0.01) and TB-a (P = 0.04), while demonstrated lower levels of b FSH (P = 0.01), perAll (P = 0.04), and h_4/h_1 (P = 0.03). The Spearman's correlation coefficient analysis revealed statistically significant correlation (P < 0.05) between TB-L, TB-b, TC-L, TC-b, perAll, perPart, h_4, h_5, t_1, h_1/t_1 and b FSH, b LH, basal endometrial thickness, LH on endometrial transition day, E_2 on endometrial transition day, P on endometrial transition day, and endometrial thickness on endometrial transition day in live birth group. The multivariate logistic regression analysis showed that the prediction Model 3 for live birth outcome [area under the curve (AUC): 0.917, 95% confidence interval (CI): 0.863 – 0.971, P < 0.001] surpassed the Model 1 (AUC: 0.698,

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95% CI: 0.593 – 0.803, P = 0.001), or the Model 2 (AUC: 0.790, 95% CI: 0.699 – 0.880, P < 0.001). The regression equations for the live birth outcomes, integrating tongue and pulse indicators with endocrine parameters, included the following measures: FSH on endometrial transition day [odds ratio (OR): 0.523, P = 0.025], LH on endometrial transition day (OR: 1.277, P = 0.029), TB-L (OR: 2.401, P = 0.001), perPart (OR: 1.018, P = 0.013), h_1 (OR: 0.065, P = 0.021), t_1 (OR: 4.354, P = 0.024), and h_4/h_1 (OR: 0.018, P = 0.016).
Conclusion In infertility patients undergoing FET, there exists a correlation between tongue and pulse indicators and endocrine parameters. The corporation of tongue and pulse indicators significantly improved the predictive capability of the model for live birth outcomes. Specifically, tongue and pulse indicators such as TB-L, perPart, h_1, t_1, and h_4/h_1 exhibited a discernible correlation with the ultimate live birth outcomes.

1 Introduction

Frozen-thawed embryo transfer (FET) has become an important part of assisted reproduction technology since its introduction over 30 years ago. Today, it is extensively used in infertility clinical treatment [1]. In a multicenter study involving 458 first-time transplant patients, the live birth rate was reported as 56.2% [2]. Even though the progress made in FET is significant, the live birth rate is not as high as it should be, indicating a need for upgrade to lift live birth rates.

In recent years, study has delved into the connection between the general health status of the human body and fertility, especially in the field of traditional Chinese medicine (TCM) physical identification and assisted reproduction [3]. The tongue and pulse indicators, serving as simple and intuitive diagnostic tools, have attracted significant attention in this regard [4, 5]. Tongue and pulse diagnosis stands as a hallmark traditional diagnostic method in TCM. Changes observed in the tongue and pulse serve as indicators reflecting physiological functions and pathological alterations in the human body, which is one of the indispensable bases for TCM diagnosis and treatment of diseases. Previous study has found a correlation between changes in the tongue and pulse characteristics and endocrine levels [6]. However, there is a lack of sufficient scientific investigation and evidence to substantiate the association between tongue and pulse characteristics and the ultimate outcomes of live births in infertile patients.

In this study, we analyzed the tongue and pulse indicators of FET patients with different live birth outcomes, explored the correlation between tongue and pulse indicators and endocrine indicators, and further investigated the intrinsic correlation between tongue and pulse indicators and live birth outcomes of patients undergoing FET. The study aims to reveal the prospective significance of tongue and pulse indicators in predicting the success rate of FET. It is anticipated to offer novel insights and strategies for clinical practices in infertility treatment while furnishing a foundational reference for TCM tongue and pulse indicators to improve the live birth outcomes among FET patients.

2 Materials and methods

2.1 Study subjects

Female infertile subjects who underwent FET to assist in conception at the Reproductive Medicine Center, Shuguang Hospital Affiliated to Shanghai University of Traditional Chinese Medicine, from March 8, 2021 to January 5, 2022, were selected for this study. The study was approved by the Medical Ethics Committee of Shuguang Hospital Affiliated to Shanghai University of Traditional Chinese Medicine (2018-626-55-02), and the clinical trial registration number is ChiCTR1900026008.

2.2 Diagnostic criteria

The diagnostic criteria for infertility are in line with relevant standards established in *Obstetrics and Gynecology* 9th edition [7] and *Guideline on Diagnosis of Infertility* [8]. Infertility is defined as the failure to conceive after a minimum of 12 months of regular sexual activity without using contraception.

2.3 Inclusion and exclusion criteria

2.3.1 Inclusion criteria The inclusion criteria for participants were as follows. (i) Women aged between 20 and 49 years experiencing infertility. (ii) Undergoing hormone replacement treatment (HRT) during FET to regulate the endometrium. (iii) Using high-quality cleavage embryos (8-cell grade II and above) or high-quality blastocysts (3BC or 3CB and above) during embryo transfer [9]. (iv) Male partners exhibiting normal results in routine semen examination. (v) Voluntarily signed the informed consent.

2.3.2 Exclusion criteria The exclusion criteria for participants were as follows. (i) Women utilizing assisted reproduction technology but not in the FET treatment stage. (ii) Women undergoing FET treatment utilizing the natural cycle program to regulate the endometrium. (iii) Presence of chromosomal abnormalities in either spouse. (iv) Concurrent chronic diseases such as hypertension, diabetes, heart disease, abnormal liver and kidney functions, coagulation disorders, cancer, or other serious life-threatening diseases incompatible with pregnancy. (v) Male partners diagnosed with severe oligozoospermia or azoospermia. (vi) Refusing to sign the informed consent. (vii) Patients not followed up to final live birth outcome. (viii) Voluntary withdrawal for personal reasons.

2.4 FET cycle program

The HRT endothelial preparation protocol was consistently employed for all participants to ensure protocol uniformity [10, 11]. Starting from day 2 – 3 of the menstrual cycle, estradiol valerate (Bayer Healthcare Co., Ltd., Germany) was orally administered at the dosage of 4 – 6 mg/d for 10 – 14 d. This regimen continued until the endometrial thickness reached an optimal state for embryo transfer, typically at least 8 mm. Subsequently, dydrogesterone (Abbott Biologicals B.V., Netherlands) was added at a dosage of 30 mg/d to facilitate endometrial transformation. Embryo transfer was conducted three days later for cleavage-stage embryos and five days later for blastocyst transfer.

2.5 Follow-up of pregnancy outcomes

Patients with intrauterine gestational sacs or ectopic pregnancies visible on vaginal ultrasound 28 d after FET were clinically confirmed pregnant [12]. A live birth is defined as the delivery of a fetus exhibiting signs of life such as breathing, heartbeat, umbilical cord pulsation, or random muscle contractions at 28 weeks into gestation or with a birth weight of 1 kg or more [13]. The clinical pregnancy rate was determined as follows: the number of clinical pregnancy cycles/the number of transfer cycles × 100%. Similarly, the live birth rate was calculated as follows: the number of live birth cycles/the number of transfer cycles × 100%.

2.6 Research grouping

According to the final outcome of live birth, the patients were divided into live birth and non-live birth groups. Patients in non-live birth group included those with unsuccessful pregnancies after FET, patients with spontaneous abortions, stillbirths, and other adverse pregnancy outcoms.

Three multivariate logistic regression models were constructed based on various datasets. Model 1 consisted of endocrine indicators only, Model 2 solely consisted of tongue and pulse indicators, and Model 3 consisted of both tongue and pulse indicators and endocrine indicators.

2.7 Data collection

2.7.1 Basic data and endocrine data collection Basic data indude the age of female participants, body mass index (BMI), the age of participants' partners, and duration of infertility. All subjects underwent to collection of the endocrine indicators during the FET cycle, including (i) collection of basic endocrine markers on day 2 – 5 into menstruation, including basic follicle stimulating hormone (b FSH), basic luteinizing hormone (b LH), basic estradiol (b E_2), basic progesterone (b P), and basic endometrial thickness. (ii) collection of endocrine markers on endometrial transition day, including FSH on endometrial transition day, LH on endometrial transition day, E_2 on endometrial transition day, P on endometrial transition day, and endometrial thickness on endometrial transition day.

2.7.2 Tongue data collection Tongue and pulse images were collected between 8:00 and 12:00 am on the day of embryo transfer to ensure data consistency. The TFDA-1 digital tongue diagnostic instrument developed by the Intelligent Diagnostic Technology Research Laboratory of Shanghai University of Traditional Chinese Medicine was employed to acquire tongue images from the subjects (Figure 1). The instrument is equipped with a stable light source with a color temperature of 5 000 K and an illuminance of 4 800 lx, providing stable and standard acquisition conditions [14]. The process of tongue image collection is as follows. (i) Following thorough communication with the subjects, the operator trained them on the proper posture for extending the tongue. Subsequently, the mouth and tongue of the subjects were meticulously cleaned to remove any food debris or foreign objects. (ii) The subjects assumed a seated position, with their lower jaw positioned against the mandibular rest within the collection ring of the TFDA-1 digital tongue diagnostic instrument. Their foreheads were near the upper end of the ring. The subjects were then instructed to close their eyes and extend their tongue relaxedly from their mouth, ensuring that the tip of the tongue hung down naturally and the tongue surface spread out. Finally, the operator clicked on the center of the screen to complete the tongue image acquisition. (iii) The subjects took a 3 – 5 min rest before extending their tongue again if their tongue images should be repeatedly captured. This helped prevent distortion of the color of the tongue body (TB) caused by prolonged tongue extension.

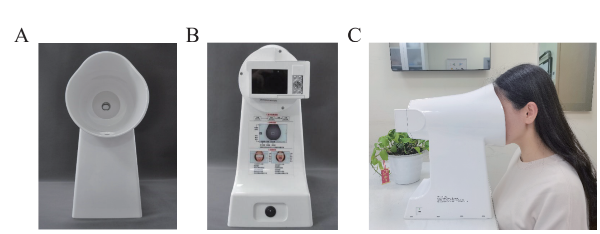

Figure 1 The TFDA-1 digital tongue diagnostic instrument

A, front view. B, back view. C, tongue image acquisition.

2.7.3 Pulse data collection The human pulse wave signals were collected using the PDA-1 single-part pulse diagnostic instrument, which was developed by the Shanghai University of Traditional Chinese Medicine (Figure 2). The process of pulse acquisition is as follows. (i) After resting for 5 – 10 min, the subjects assumed a seated position facing the collector. They relaxed their left forearm and stretched it out in front of them, placing their wrist on the pulse pillow with the palm facing upward. Their elbow was flexed at a 120° angle, and they maintained an upright posture while breathing steadily. (ii) The probe of the PDA-1 single-part pulse diagnostic instrument was placed on the left hand of subjects to collect the pulse map. The best pulse map was collected for 30 s after the waveform became smooth and the wave amplitude maximum. (iii) During the pulse wave collection process, the subjects were required not to talk or move their bodies in order not to affect the accuracy of the pulse wave.

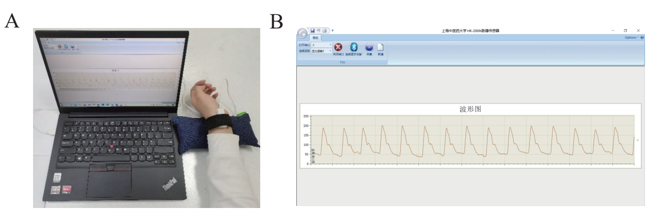

Figure 2 PDA-1 single-part pulse diagnostic instrument
A, pulse wave acquisition. B, acquisition software interface.

2.8 Tongue and pulse indicators analysis

Tongue images were primarily analyzed from color space parameters and tongue coating (TC) parameters. This analysis was conducted using the "TCM Tongue Diagnosis Analysis System (TDAS) V2.0" developed by the Research Center of Information Science and Technology of Traditional Chinese Medicine of Shanghai University of Traditional Chinese Medicine [15]. The tongue features were extracted automatically (Figure 3). The segmentation of the tongue body and tongue coating regions was accomplished through the split and merge algorithm in conjunction with the color threshold method [16, 17].

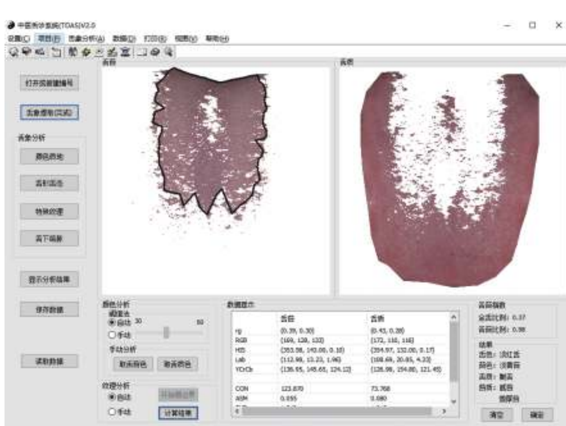

Figure 3 The interface of the TDAS V2.0 tongue image analysis software

The Lab color space is a homogeneous color space, a color system based on physiological features [15]. In the Lab color space, the L value denotes lightness, where a higher value indicates greater brightness. The a value is presented by the red-green axis, and positive value is in association with red color, with higher value indicating redder color, while the negative value with the green color, with lower value indicating greener color. The b value corresponds to the yellow-blue axis in the Lab color space. The positive value is related to the yellow color, the higher the value, the yellower the color is; while the negative value to the blue color, with lower value denoted by bluer color, as shown in Figure 4 [15]. The primary parameters include TB color characteristic parameters (TB-L, TB-a, and TB-b) and TC color characteristic parameters (TC-L, TC-a, and TC-b). perAll and perPart are the TC parameters, and perAll is the ratio of the pixel area of the TC to that of the whole tongue, with higher value indicating thicker TC; perPart holds a diagnostic value for thin coating, with a larger value denoting for thinner TC [18].

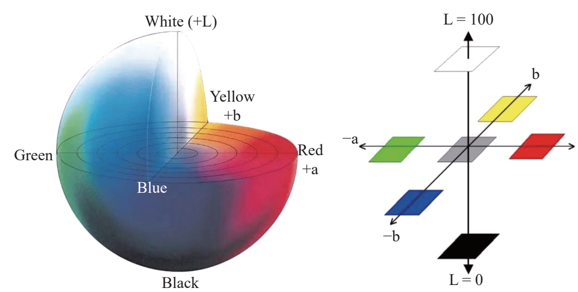

Figure 4 Schematic diagram of the Lab color space

The pulse wave indicators were extracted by pulse analysis software (PulseCol), and their significance was analyzed using the time domain analysis method, including h_1, h_4, h_5, t_1, h_1/t_1, and h_4/h_1 (Figure 5).

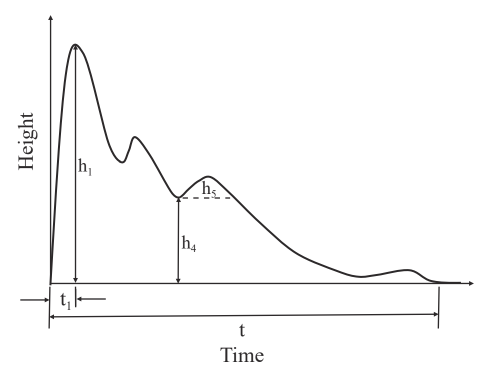

Figure 5 Schematic diagram of the pulse wave indicator

h_1 is the amplitude of the main wave, mainly reflecting the ejection function of the left ventricle; h_4 is the amplitude of the descending isthmus, mainly reflecting the state of peripheral resistance of the arterial vasculature; h_5 is the amplitude of the repetitive wave, mainly reflecting the compliance of the large arteries; t_1 is the value of the time between the starting point of the pulse map and the peak point of the main wave, mainly reflecting the length of the left ventricle's rapid ejection period; h_1/t_1 primarily reflects the strength of the cardiovascular function; h_4/h_1 is the ratio of descending isthmus amplitude to main wave amplitude, which mainly reflects the peripheral resistance [19].

2.9 Statistical analysis

Measurement data in the study were presented as mean ± standard deviation (SD). If the data conformed to normality and chi-square distribution, the comparison between the two groups was conducted using independent samples *t* test. Conversely, if the data did not conform to normality and chi-square distribution, the comparison between the two groups was performed using Wilcoxon rank-sum test. Spearman's correlation analysis was carried out to assess the correlation between tongue and pulse indicators, and endocrine parameters in the two groups. Subsequently, multivariate logistic regression was employed to analyze the influencing factors of live birth outcomes and establish a categorical model. The diagnostic efficacy was analyzed with the receiver operating characteristic (ROC) curve. The statistical analysis was performed using SPSS 26.0 software, and the correlation heatmap was drawn using Origin 2022 software. The difference was considered statistically significant when $P < 0.05$.

3 Results

3.1 Comparison of basic information and endocrine parameters

A total of 420 patients were recruited in this study. However, only 222 remained following the inclusion and exclusion criteria, among whom 78 were divided into live birth group and 144 to non-live birth group.

As indicated in Table 1, the age and b FSH of women in live birth group were significantly lower than those in non-live birth group ($P < 0.05$). However, the comparison of the remaining indicators between the two groups did not yield statistically significant differences ($P > 0.05$).

3.2 Comparison of tongue and pulse indicators

Live birth group exhibited significantly higher levels of TB-L and TB-a, and lower levels of perAll and h_4/h_1 compared with non-live birth group ($P < 0.05$, Table 2)

3.3 Spearman's correlation analysis

3.3.1 Correlation between tongue and pulse indicators and endocrine parameters in live birth group The results showed that in live birth group, TB-L was positively correlated with basal endothelial thickness and E_2, and negatively correlated with P on endothelial transition day; TB-b was positively correlated with b FSH, b LH, and LH on endothelial transition day, and negatively correlated with basal endothelial thickness; TC-L was positively correlated with E_2, and negatively correlated with LH and P on endothelial transition day; TC-b was positively correlated with b FSH, b LH and LH on endothelial transition day; perAll was negatively correlated with b FSH and b LH; perPart was positively correlated with basal endothelial thickness and negatively correlated with b FSH and b LH; h_4 was positively correlated with b LH and negatively correlated with endothelial thickness on endothelial transition day; h_5 was positively correlated with P and negatively correlated with E_2 on endothelial transition day; t_1 was positively correlated with basal endothelial thickness; h_1/t_1 was negatively correlated with endothelial thickness on endothelial transition day. The specific correlation coefficients are shown in Table 3, and the heatmap is shown in Figure 6A.

3.3.2 Correlation between tongue and pulse indicators and endocrine parameters in non-live birth group The results showed that in non-live birth group, TB-L was negatively correlated with b LH; TB-a and TB-b were

Table 1 Comparison of basic information and endocrine parameters between live birth and non-live birth groups ($\bar{x} \pm s$)

Group	Age of female participants (years)	BMI (kg/m²)	Age of the participants' partners (years)	Year of infertility (years)
Live birth (*n* = 78)	31.33 ± 4.37	23.72 ± 4.01	33.86 ± 4.75	3.23 ± 1.73
Non-live birth (*n* = 144)	33.43 ± 5.49	23.48 ± 3.94	34.64 ± 5.89	3.56 ± 2.34
P value	< 0.01	0.73	0.29	0.27

Group	b FSH (IU/L)	b LH (IU/L)	b E_2 (pmol/L)	b P (nmol/L)	Basal endometrial thickness (mm)
Live birth (*n* = 78)	5.36 ± 1.89	5.14 ± 3.07	118.99 ± 22.19	1.56 ± 4.19	6.06 ± 1.48
Non-live birth (*n* = 144)	7.80 ± 4.35	5.77 ± 2.86	166.08 ± 22.56	0.95 ± 1.62	5.84 ± 1.65
P value	0.01	0.33	0.05	0.33	0.37

Group	FSH on endometrial transition day (IU/L)	LH on endometrial transition day (IU/L)	E_2 on endometrial transition day (pmol/L)	P on endometrial transition day (nmol/L)	Endometrial thickness on endometrial transition day (mm)
Live birth (*n* = 78)	4.11 ± 2.24	9.45 ± 6.58	1 223.81 ± 19.17	0.83 ± 2.38	10.27 ± 2.28
Non-live birth (*n* = 144)	4.61 ± 4.36	8.75 ± 10.77	1 945.77 ± 49.98	0.84 ± 3.55	9.88 ± 2.02
P value	0.29	0.57	0.24	0.98	0.20

Table 2 Comparison of tongue image and pulse wave parameters between live birth and non-live birth groups ($\bar{x} \pm s$)

Group	TB-L	TB-a	TB-b	TC-L	TC-a
Live birth (n = 78)	47.82 ± 2.46	28.27 ± 2.05	8.62 ± 2.01	46.26 ± 5.74	20.16 ± 2.07
Non-live birth (n = 144)	46.29 ± 2.35	27.74 ± 2.16	8.62 ± 2.11	46.63 ± 5.28	19.97 ± 1.98
P value	0.01	0.04	0.99	0.63	0.50

Group	TC-b	perAll	perPart	h_1	h_4
Live birth (n = 78)	5.33 ± 2.03	0.27 ± 0.11	0.81 ± 0.40	11.75 ± 3.53	3.08 ± 1.38
Non-live birth (n = 144)	5.28 ± 2.26	0.30 ± 0.10	0.87 ± 0.67	11.61 ± 3.43	3.30 ± 1.24
P value	0.88	0.04	0.41	0.79	0.27

Group	h_5	t_1	h_1/t_1	h_4/h_1
Live birth (n = 78)	0.39 ± 0.41	0.13 ± 0.01	3.77 ± 1.12	0.26 ± 0.08
Non-live birth (n = 144)	0.42 ± 0.47	0.12 ± 0.01	3.79 ± 1.10	0.29 ± 0.09
P value	0.58	0.36	0.94	0.03

Table 3 Spearman's correlation analysis of tongue and pulse indicators with endocrine indicators in live birth group (n = 78)

Parameter	b FSH	b LH	b E_2	b P	Basal endometrial thickness	FSH on endometrial transition day	LH on endometrial transition day	E_2 on endometrial transition day	P on endometrial transition day	Endometrial thickness on endometrial transition day
TB-L	– 0.250	– 0.130	– 0.040	– 0.100	0.320*	– 0.010	– 0.030	0.300*	– 0.330**	0.11
TB-a	0.280	0.070	0.140	– 0.020	– 0.110	– 0.150	– 0.070	– 0.150	0.010	0.080
TB-b	0.500**	0.400**	0.010	– 0.110	– 0.300*	0.170	0.380**	0.080	– 0.070	– 0.050
TC-L	– 0.270	– 0.270	0.060	– 0.160	0.150	– 0.180	– 0.250*	0.280*	– 0.330**	0.160
TC-a	0.130	0.030	– 0.140	0.160	– 0.050	– 0.040	– 0.190	– 0.040	0.120	– 0.040
TC-b	0.450**	0.420**	– 0.020	– 0.070	– 0.170	0.160	0.370**	0.160	– 0.120	– 0.150
perAll	– 0.390**	– 0.320*	– 0.080	0.060	0.180	0.070	– 0.100	0.000	0.000	0.060
perPart	– 0.290*	– 0.320*	0.250	0.170	0.320*	0.070	– 0.020	0.160	– 0.190	0.020
h_1	0.077	0.259	– 0.305	0.117	0.202	0.101	0.146	– 0.106	0.110	– 0.249
h_4	0.214	0.321*	– 0.239	0.073	– 0.048	0.069	0.086	0.062	– 0.003	– 0.274*
h_5	0.031	0.084	0.067	0.109	0.005	0.139	– 0.019	– 0.362**	0.276*	0.045
t_1	– 0.023	0.179	– 0.040	– 0.090	0.405**	– 0.012	0.159	0.102	– 0.187	0.219
h_1/t_1	0.140	0.220	– 0.220	0.080	0.010	0.150	0.090	– 0.150	0.190	– 0.320*
h_4/h_1	0.080	0.050	– 0.100	– 0.010	– 0.240	– 0.090	– 0.120	0.190	– 0.190	– 0.130

The values in Table 3 are correlation coefficients (r). The asterisks (*) indicate significant correlation (absolute value of $r > 0.2$ and $P < 0.05$), and the asterisks (**) significant correlation (absolute value of $r > 0.3$ and $P < 0.01$).

positively correlated with b LH; perAll was negatively correlated with b FSH and b LH; h_1 was positively correlated with b LH and b E_2; h_4 was positively correlated with E_2 on endothelial transition day; h_5 was positively correlated with b E_2; t_1 was negatively correlated with b FSH and endothelial thickness on endometrial transition day; h_1/t_1 was positively correlated with b E_2; h_4/h_1 was positively correlated with E_2 on endothelial transition day, and negatively correlated with b LH. The specific correlation coefficients are shown in Table 4, and the heatmap is shown in Figure 6B.

3.4 Multivariate logistic regression analysis of factors influencing the outcome of live births

Multivariate logistic regression was performed with live birth outcomes serving as the dependent variable, while tongue and pulse indicators along with endocrine indicators as independent variables.

The results showed that the area under the curve (AUC) of Model 1 was 0.698 [95% confidence interval (CI): 0.593 – 0.803, $P < 0.01$], and the parameter in Model 1 was b FSH ($P < 0.05$); the AUC of Model 2 was 0.790

Table 4 Spearman's correlation coefficient analysis of tongue and pulse indicators with endocrine indicators in non-live birth group (n = 144)

Parameter	b FSH	b LH	b E_2	b P	Basal endometrial thickness	FSH on endometrial transition day	LH on endometrial transition day	E_2 on endometrial transition day	P on endometrial transition day	Endometrial thickness on endometrial transition day
TB-L	0.050	– 0.200*	– 0.040	– 0.020	0.140	0.090	0.090	0.100	– 0.140	0.000
TB-a	0.170	0.360**	0.020	0.040	– 0.080	– 0.020	0.040	0.000	– 0.060	– 0.120
TB-b	0.090	0.200*	0.020	0.030	– 0.150	0.050	– 0.040	– 0.100	– 0.100	0.040
TC-L	0.010	– 0.100	0.080	– 0.070	0.030	0.010	0.070	0.140	– 0.020	– 0.020
TC-a	0.170	0.100	– 0.070	0.040	– 0.010	0.080	0.160	0.100	– 0.030	0.050
TC-b	0.010	0.050	0.030	0.010	– 0.050	0.060	– 0.020	– 0.020	– 0.090	0.060
perAll	– 0.230*	– 0.340**	0.000	0.060	0.110	– 0.140	– 0.090	0.070	0.130	0.060
perPart	– 0.060	– 0.060	– 0.080	0.130	0.060	0.010	– 0.010	– 0.080	0.090	– 0.130
h_1	0.040	0.253*	0.224*	– 0.066	– 0.145	– 0.101	– 0.077	0.145	0.087	0.023
h_4	– 0.014	– 0.030	0.148	0.096	– 0.069	– 0.094	– 0.092	0.357**	0.115	– 0.113
h_5	– 0.179	0.077	0.221*	– 0.033	0.096	– 0.013	0.019	– 0.095	– 0.002	0.145
t_1	– 0.249*	0.042	– 0.064	– 0.102	– 0.183	0.014	0.094	0.043	– 0.038	– 0.227*
h_1/t_1	0.100	0.200	0.270*	– 0.030	– 0.040	– 0.140	– 0.150	0.170	0.090	0.100
h_4/h_1	0.030	– 0.230*	– 0.080	0.160	– 0.060	– 0.030	– 0.040	0.180*	0.040	– 0.160

The values in Table 4 are correlation coefficients (r). The asterisks (*) indicate significant correlation (absolute value of r > 0.2 and P < 0.05), and the asterisks (**) significant correlation (absolute value of r > 0.3 and P < 0.01).

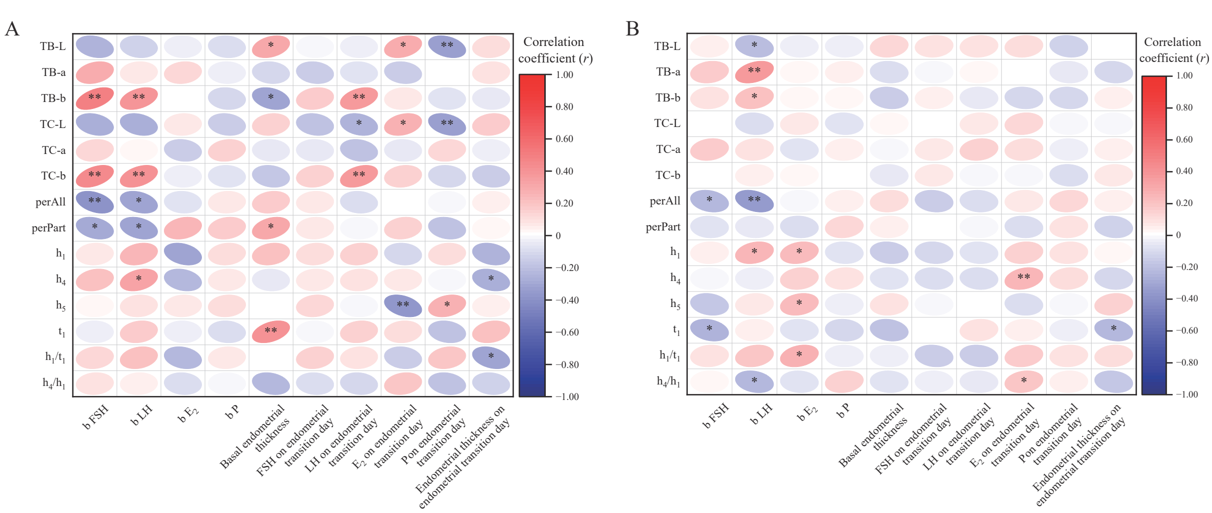

Figure 6 Spearman's correlation between tongue and pulse indicators and endocrine indicators in live birth and non-live birth groups

A, live birth group. B, non-live birth group. Red squares indicate a positive correlation (r > 0), blue squares negative correlation (r < 0). The asterisks (*) indicate significant correlation (absolute value of r > 0.2 and P < 0.05), and the asterisks (**) significant correlation (absolute value of r > 0.3 and P < 0.01).

(95% CI: 0.699 – 0.880, P < 0.001), and the parameter in Model 2 was TB-L (P < 0.05); the AUC of Model 3 was 0.917 (95% CI: 0.863 – 0.971, P < 0.001), and the indicators in Model 3 were FSH on endothelial transition day, LH on endothelial transition day, TB-L, perPart, h_1, t_1, h_4/h_1 (P < 0.05) (Table 5 and 6, and Figure 7).

Table 5 Multivariate logistic regression analysis of factors influencing live birth outcomes

Model	Parameter	β	SE	Wald	OR	95% CI	*P* value
Model 1	b FSH	– 0.188	0.089	4.525	0.828	0.696 – 0.985	0.033
	b LH	0.003	0.007	0.182	1.003	0.990 – 1.016	0.669
	b E_2	– 0.002	0.002	1.403	0.998	0.994 – 1.001	0.236
	b P	0.131	0.077	2.943	1.140	0.981 – 1.325	0.086
	Basal endometrial thickness	– 0.003	0.135	0.000	0.997	0.765 – 1.301	0.985
	FSH on endometrial transition day	0.024	0.105	0.054	1.025	0.833 – 1.260	0.816
	LH on endometrial transition day	0.002	0.034	0.004	1.002	0.938 – 1.070	0.950
	E_2 on endometrial transition day	0.000	0.000	0.032	1.000	1.000 – 1.000	0.859
	P on endometrial transition day	0.019	0.056	0.118	1.019	0.913 – 1.138	0.732
	Endometrial thickness on endometrial transition day	0.103	0.117	0.765	1.108	0.880 – 1.395	0.382
Model 2	TB-L	0.246	0.121	4.097	1.278	1.008 – 1.622	0.043
	TB-a	0.144	0.166	0.749	1.155	0.834 – 1.599	0.387
	TB-b	– 0.375	0.226	2.759	0.687	0.441 – 1.070	0.097
	TC-L	– 0.070	0.058	1.476	0.932	0.833 – 1.044	0.224
	TC-a	– 0.071	0.120	0.355	0.931	0.736 – 1.178	0.551
	TC-b	0.097	0.190	0.260	1.102	0.759 – 1.598	0.610
	perAll	– 4.298	4.389	0.959	0.014	0.000 – 73.967	0.327
	perPart	– 0.179	0.346	0.269	0.836	0.425 – 1.646	0.604
	h_1	– 0.308	0.447	0.475	0.735	0.306 – 1.764	0.491
	h_4	0.278	0.698	0.159	1.321	0.337 – 5.185	0.690
	h_5	– 1.066	0.653	2.662	0.345	0.096 – 1.239	0.103
	t_1	2.204	4.362	0.465	1.773	0.000 – 2.857	0.495
	h_1/t_1	0.664	1.366	0.236	1.942	0.133 – 28.26	0.627
	h_4/h_1	– 10.977	7.333	2.241	0.000	0.000 – 29.813	0.134
Model 3	b FSH	– 0.215	0.183	1.375	0.807	0.563 – 1.155	0.241
	b LH	0.004	0.008	0.179	1.004	0.987 – 1.020	0.672
	b E_2	– 0.002	0.004	0.340	0.998	0.989 – 1.006	0.560
	b P	0.134	0.136	0.974	1.144	0.876 – 1.493	0.324
	Basal endometrial thickness	– 0.132	0.245	0.290	0.876	0.542 – 1.417	0.590
	FSH on endometrial transition day	– 0.649	0.289	5.040	0.523	0.297 – 0.921	0.025
	LH on endometrial transition day	0.245	0.112	4.793	1.277	1.026 – 1.590	0.029
	E_2 on endometrial transition day	0.000	0.000	1.020	1.000	0.999 – 1.000	0.313
	P on endometrial transition day	0.113	0.077	2.142	1.120	0.962 – 1.304	0.143
	Endometrial thickness on endometrial transition day	0.110	0.214	0.261	1.116	0.733 – 1.698	0.609
	TB-L	0.876	0.272	10.373	2.401	1.409 – 4.091	0.001
	TB-a	– 0.146	0.319	0.211	0.864	0.462 – 1.613	0.646
	TB-b	– 0.346	0.470	0.541	0.708	0.282 – 1.777	0.462
	TC-L	– 0.101	0.118	0.729	0.904	0.718 – 1.139	0.393
	TC-a	0.243	0.317	0.588	1.276	0.685 – 2.376	0.443
	TC-b	– 0.024	0.375	0.004	0.977	0.468 – 2.038	0.950
	perAll	– 7.234	10.056	0.517	0.001	0.000 – 2.619	0.472
	perPart	2.321	0.937	6.138	1.018	1.624 – 6.385	0.013
	h_1	– 2.733	1.183	5.341	0.065	0.006 – 0.660	0.021
	h_4	3.080	1.914	2.590	2.176	0.511 – 9.257	0.108
	h_5	– 0.670	1.535	0.190	0.512	0.025 – 10.369	0.663
	t_1	2.525	1.118	5.099	4.354	2.980 – 6.370	0.024
	h_1/t_1	5.906	3.461	2.912	3.673	0.416 – 3.246	0.088
	h_4/h_1	– 4.000	1.870	4.576	0.018	0.000 – 0.715	0.016

β (Beta): regression coefficient represents the relationship between the predictor variables and the outcome variable. SE (standard error): SE of the estimate, indicates the variability or uncertainty in the regression coefficient. Wald: Wald statistic is a measure used for testing the significance of the coefficients in the logistic regression model. OR: OR measures the association between the predictor variables and the outcome variable. 95% CI: 95% CI provides a range within which the true value of the parameter is likely to fall. *P*: *P* value determines the significance of the regression coefficients and is a measure of the probability that the observed results occurred by chance.

Table 6 Evaluation for the live birth predictive models

Model	AUC	SE	95% CI	*P* value
Model 1	0.698	0.054	0.593 – 0.803	0.001
Model 2	0.790	0.046	0.699 – 0.880	< 0.001
Model 3	0.917	0.028	0.863 – 0.971	< 0.001

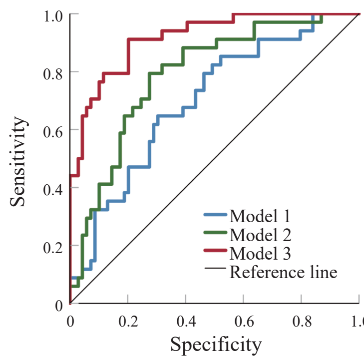

Figure 7 ROC of the live birth predictive models

4 Discussion

Reproductive disorders have emerged as a global concern, affecting populations worldwide. The incidence of infertility in China has surged to 18% and continues to rise [20]. FET typically involves multiple cycles before achieving a live birth [21], leading to significant financial expenses and posing substantial challenges to the mental health and quality of life of infertile couples. Therefore, it is crucial for infertile couples to access a convenient, cost-effective, and accurate method to predict the outcome of FET live birth, a method that can assist them in making informed decisions after carefully considering the benefits and risks involved.

The study revealed that in comparison with non-live birth group, live birth group had higher tongue brightness, redder color, and thinner TC, and lower peripheral vascular resistance. HUANG et al. [22] reported that after taking tonifying Qi and blood medicine, the I and L values of TB decreased significantly, while the s and a values of TB increased significantly, indicating a reduction in the brightness of TB and a tongue color alteration from light white to light red after the medical intervention. The balance between Qi and blood is crucial for normal reproductive function in females. The tongue, reflecting the microcirculation of the human body, should appear red and bright. Such an appearance is an important indicator of the body's harmonization of Yin and Yang, and the smooth flow of Qi and blood. The results of this study also demonstrated the significance of smooth Qi and blood circulation on the day of embryo transfer, and the importance of a favorable pulse path to the ultimate outcome of live birth in infertile women.

The present study delved deeper into the correlation between tongue and pulse characteristics and reproductive endocrinology. It was found that tongue and pulse indicators exhibited varying degrees of correlation with endocrine indicators and endometrial thickness in both live birth and non-live birth groups. The correlation between tongue and pulse characteristics and both endocrine indicators and endometrial thickness was found to be more significant in live birth group compared with non-live birth group. The color of the tongue is influenced by the body's blood circulation and varies with changes in body temperatures, hormones, and hemodynamic parameters [23]. The pulse is an external manifestation of the internal environment, reflecting the cardiovascular function of the human body and the blood perfusion of organs [24-26]. The changes in pulse are closely related to peripheral vascular resistance and arterial compliance. When estrogen levels peak in the menstrual cycle, women experience a significant enhancement in cardiac function, an increase in excretion, and a reduction in peripheral vascular resistance to their lowest levels [27]. In addition, research has shown that besides the effects of estrogen, progesterone also plays a role in dilating blood vessels and reducing peripheral resistance [28]. In the clinical diagnosis and treatment of infertility, the tongue and pulse indicators are important information sources for syndrome differentiation and treatment in TCM. It is a "mirror" to evaluate the changes in women's health status [29]. The dynamic changes in tongue and pulse are closely related to the periodic changes in endocrine [22, 30].

Multivariate logistic regression analysis revealed that the tongue and pulse indicators TB-L, perPart, h_1, t_1, and h_4/h_1 could be included in the regression equation for predicting the outcome of live birth, suggesting that the above indicators can be used as potential indicators for assessing the outcome of live birth. Compared with the prediction model employing endocrine parameters alone (Model 1) or tongue and pulse parameters alone (Model 2), the Model 3 that integrated the two parameters was the most effective in assessing the outcome of live birth (AUC = 0.917), suggesting the effectiveness of combining the tongue and pulse parameters with endocrine parameters in predicting the outcome of live birth in patients experiencing FET. The integration of tongue and pulse indicators with endocrine parameters can provide a comprehensive reflection of the state of infertile women on the day of embryo transfer and assess the outcome of live birth from various aspects, including the balance between body's Qi and blood, the functional strength of internal organs, and the progression or regression of health conditions. By analyzing these factors, clinicians can gain deeper insights into the overall condition of the patient and better predict the outcome of the transfer at an early stage, so as to reduce the burden of medical care.

This study uses contemporary tongue and pulse diagnostic technologies, and utilizes quantitative data analysis to clearly demonstrate the relationship between tongue and pulse indicators and endocrine parameters of women experiencing different live birth outcomes following FET. The study discovered that tongue and pulse indicators contribute meaningfully to the classification and prediction of live birth outcomes in patients undergoing artificial cycle FET, which provides a practical groundwork and data support for the application of tongue and pulse indicators in the clinical efficacy evaluation of infertility treatments. Additionally, this study also has shortcomings, such as the small sample size and its cross-sectional feature. Therefore, in future studies, we will consider expanding the sample size, adding the time factor, and continuously and dynamically observing the specific relationship between the tongue and pulse indicators and the outcome of live birth in infertile women.

5 Conclusion

In this study, we analyzed the correlation between tongue and pulse indicators and endocrine parameters in females presenting different live birth outcomes and constructed logistic classification and prediction models in this regard. We found that the integration of tongue and pulse image parameters could significantly improve the efficacy of the model in predicting live birth outcomes among patients undergoing FET. This confirmed the value of tongue and pulse parameters in the prediction of FET live birth outcomes with objective data, providing a basis for TCM tongue and pulse diagnostics to improve the live birth outcome in patients undergoing FET.

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Competing interests

The authors declare no conflict of interest.

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冻融胚胎移植中舌象和脉象指标与活产结局的相关性研究

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【**摘要**】**目的** 探讨冻融胚胎移植 (FET) 患者舌象和脉象指标与活产结局的相关性，及其与患者内分泌指标的关联性。**方法** 本研究于 2021 年 3 月 8 日 – 2022 年 1 月 5 日在上海中医药大学附属曙光医院生殖医学中心进行。根据活产结局将 FET 患者分为活产组和非活产组。分析两组的内分泌指标 [基础促卵泡生成素 (b FSH)、基础促黄体生成素 (b LH)、基础雌二醇 (b E_2)、基础孕酮 (b P)、基础子宫内膜厚度、内膜转换日促卵泡生成素 (FSH)、内膜转换日促黄体生成素 (LH)、内膜转换日雌二醇 (E_2)、内膜转换日孕酮 (P)、内膜转换日子宫内膜厚度] 与舌象和脉象指标 [舌质 (TB)-L, TB-a, TB-b, 舌苔 (TC)-L, TC-a, TC-b, perAll, perPart, h_1, h_4, h_5, t_1, h_1/t_1, h_4/h_1] 之间的差异，并用 Spearman 相关系数分析以上变量之间的相关性。采用多元 logistic 回归分析方法，筛选基于不同数据集的活产预测模型中的影响因素，包括仅由内分泌指标组成的模型 1、仅由舌象和脉象指标组成的模型 2、由舌象和脉象指标以及内分泌指标组成的模型 3，并对不同数据集模型的有效性进行评价。**结果** 研究纳入活产组 78 例，非活产组 144 例。与非活产组相比，活产组的 TB-L (P = 0.01) 和 TB-a (P = 0.04) 升高，b FSH (P = 0.01)、perAll (P = 0.04) 和 h_4/h_1 (P = 0.03) 降低。Spearman 相关分析显示，活产组的 TB-L、TB-b、TC-L、TC-b、perAll、perPart、h_4、h_5、t_1、h_1/t_1 与 b FSH、b LH、基础子宫内膜厚度、内膜转换日 LH、内膜转换日 E_2、内膜转换日 P、内膜转换日子宫内膜厚度的相关性均有统计学意义（$P < 0.05$）。多元 logistic 回归分析显示，模型 3 [曲线下面积（AUC）：0.917，95% 置信区间（CI）：0.863 – 0.971，$P < 0.001$] 优于模型 1（AUC：0.698，95% CI：0.593 – 0.803，P = 0.001）和模型 2（AUC：0.790，95% CI：0.699 – 0.880，$P < 0.001$）。将舌象和脉象指标与内分泌指标相结合，建立活产结局的回归方程，最终纳入以下指标: 内膜转换日 FSH [比值比 (OR): 0.523, P = 0.025]、内膜转换日 LH (OR: 1.277, P = 0.029)、TB-L (OR: 2.401, P = 0.001)、perPart (OR: 1.018, P = 0.013)、h_1 (OR: 0.065, P = 0.021)、t_1 (OR: 4.354, P = 0.024)、h_4/h_1 (OR: 0.018, P = 0.016)。**结论** 在接受 FET 治疗的不孕症患者中，舌象和脉象指标与内分泌指标之间存在相关性。舌象和脉象指标的加入显著提高了模型对活产结局的预测能力。具体而言，舌象和脉象指标 TB-L、PerPart、h_1、t_1 和 h_4/h_1 与最终的活产结局有明显的相关性。

【**关键词**】舌象图像；脉搏波；活产结局；冻融胚胎移植；多元 logistic 回归