# Perfect pregnancy camp report

# the perfect pregnant mother training camp project introduction

Project time:

Phase one: October 3-7, 2018 phase two: October 11-15, 2018

Five groups of families were invited to participate in two sessions of the five-day camp, which included accommodation, three meals a day (plus meals), customized courses and keeping biological samples.

#### 1 Team introduction

the project team is composed of multi-disciplinary medical staff of Peking union medical college hospital, **Seven Tian Zhen team(**七田真团队) of being yoga and fetal education teacher during pregnancy , and **Nie Qiaole team** (聂巧乐团队) *of* music childbirth course. Professor Ma Liangkun is the project leader.

- 1) Peking union medical college hospital team: responsible for project planning, operation, specimen collection and processing, data collection, data analysis, professional course design, teaching and other contents.
- 2) Seven Tian Zhen team: responsible for teaching yoga and fetal education during pregnancy.
  - 3) Nie Qiaole team: responsible for the teaching of music childbirth course.

No	Team member	Department	Duty
1	马良坤	Obstetrics	Project principal
2	聂敏	Endocrinology	Project planning, specimen storage
3	刘鹏举	Nutrition	Nutrition management, teaching
4	段艳平	Psychology	Mental health management, teaching
5	李倩	Stomatology	Pregnancy oral care
6	刘虹	Obstetrics	Safe labor, breastfeeding
7	张素菡	Obstetrics	Project planning, management, execution
8	林航	Obstetrics	Project planning
9	李曼玉	Obstetrics	Specimen transfer
10	田莹	Obstetrics	Project planning
11	刘洁颖	Central lab	Partial specimen testing
12	李洪雷	Clinical lab	Specimen test, partial specimen storage

No	Team	Item	Content
1	七田真团队	Pregnancy Yoga	60-minute comprehensive course during pregnancy Breathing and body exercises, adjusting posture, the course design is rigorous and full of fun, relieves the symptoms of discomfort and is conducive to the physical and mental health of pregnant women.
2	聂巧乐团队	Pregnancy music	How to scientifically interact with the fetus?  How to use the five-dimensional music breathing analgesia to reduce the pain of childbirth?  How to make music self-hypnosis during childbirth?

#### 2. Course introduction

### 1) medical staff courses include:

safety delivery, lectured by Ma Liangkun, Liu Hong and Li Manyu. Teaching content: labor process, labor preparation, contractions pain response, vaginal delivery and cesarean section, delivery room story sharing. Through knowledge review and simulated delivery process, pregnant mothers and their families can understand the birth process and get ready for that.

Breastfeeding, taught by Li Rui. Lecture: the benefits of breastfeeding, breastfeeding posture, common problems and pitfalls of breastfeeding.

**Nutrition**, teaching teacher: Liu Pengju, teaching content: weight management during pregnancy, nutritional needs during pregnancy, common dietary errors.

**Psychology** during pregnancy, teachers: Ma Liangkun, Duan Yanping, teaching content: group counseling, depression during pregnancy, anxiety, postpartum depression, family relationship. **Mother and infant oral care**, teaching teacher: Zhang Xin, teaching content: the importance of mother and infant oral health, how to carry out oral care, common oral problems.

### 2) pregnancy yoga classes

Instructor: **Seven Tian Zhen** teacher of yoga during pregnancy

After 14 weeks of pregnancy, the mother may experience a variety of uncomfortable symptoms throughout the pregnancy, such as lumbosacral pain, edema, frequent urination, heartburn and other problems. In the 60-minutes comprehensive course, the instructor will conduct specialized courses according to different symptoms, and adjust the posture through breathing and asana exercises. The course design is rigorous and full of fun, which can alleviate uncomfortable symptoms and benefit the physical and mental health of pregnant women.

### 3) fetal education courses

Teacher: **Seven Tian Zhen** prenatal education teacher

course topic: the mystery of the right brain, a whole feeling of mother and fetal, make a vow to the baby, the language of sunshine, return to the womb, holy lotus flower, love communication, friendly family.

introduce: the effectiveness of communication by using the brain of the right brain work area , help the mothers to experience and feel how does their right brain work area work, and practice to send messages and communicate with their fetus through the right brain way

through recognition of fetal ability and good interactive communication with their fetus, put their good wishes for the future to the fetus, and later in real achieve it.

Through the mother and child transposition thinking, let mother experience in fetal period, the baby's various needs, and please mother to meet the need of their fetus.

Through the practice of the imagination of three scene, give the mother a map and compass the confidence of the safety delivery to the mother.

help build smooth effective communication mode between husband and wife, and a happy warm living environment for the mother.

help to establish the beliefs of breastfeeding during pregnancy, and scientific concept of the *yuezi* period.

### 4) music course

Church of pregnant mothers with scientific way and the fetus parent-child connection and interaction with

music combined with the techniques, such as breathing, mindfulness ,hypnosis, face of the contractions in a scientific way , effective remission delivery pain of the childbirth.

with music and hypnosis can be deep relax in the face of the first labor, effectively save the physical power.

Scientific pelvic swing, effectively reduce the labor pain, shorten labor time

with music, soft touch can effectively stimulate the secretion of endorphins, remission delivery pain

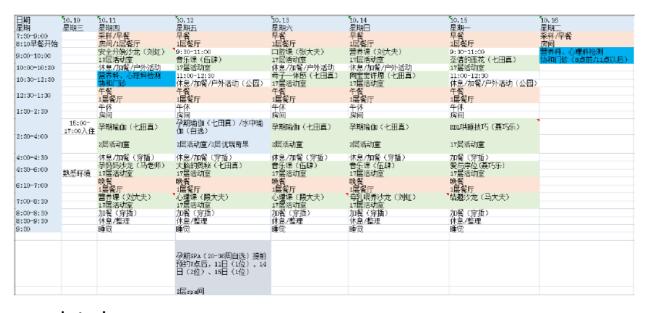
scientific use of lullaby combined with breathing, help the newborn to have a better mood and be calm , also learn some sleep tips

# 5) class schedule display

# The first phase:

	74.						
	10.2	10.3	10.4	10.5	10.6	10.7	10.8
星期		星期三	星期四	星期五	星期六	星期日	星期一
7:30-9:00		采样/早餐	早餐	早餐	早餐	早餐	菜样/早 <b>餐</b>
8:00早餐开始		房间/1层餐厅	1层餐厅	1层餐厅	1层餐厅	1层餐厅	房间
9:00-10:00		安全分娩沙龙(刘虹)	9:30-11:00	母乳喂养沙龙(刘 虹)	营养课 (刘大夫)	9:30-11:00	
		17层活动室	音乐课(伍肆)	17层活动室	17层活动室	音乐课 (伍肆)	
10:00-10:30		休息/加餐/户外活动	17层活动室	休息/加餐/户外活动	休息/加餐/户外活动	17层活动室	
10:30-12:00		音乐课〔伍肆〕	11:00-11:30	音乐课(伍肆)	音乐课(伍肆)	11:00-11:30	
10.50-12.00		2/17层活动室	休息/加餐/户外活动	17层活动室	17层活动室	休息/加餐/户外活动	
12:00-1:00		午餐	午餐	午餐	午餐	午餐	
12.00 1.00		1层餐厅	1层餐厅	1层餐厅	1层餐厅	1层餐厅	
1:00-2:00		午休	午体	午休	午体	午休 房间	
1100 1100		房间	房间	房间	房间	房间	
2:00-4:00	14:00- 17:00入住	孕期瑜伽 (七田真)	孕期瑜伽(七田真)	孕期瑜伽〔七田真〕/ 水中瑜伽〔自选〕	孕期瑜伽 (七田真)	RBL哄睡技巧(聂巧乐)	
2.00		2层活动室	2层活动室	2层活动室/2层优瑞奇 果	2层活动室	17层活动室	
4:00-4:30		休息/加餐(穿插)	休息/加餐(穿插)	休息/加餐(穿插)	休息/加餐(穿插)	加餐(穿插)	
4:30-6:00	熟悉环境	户外活动(妈咪爱) 公园	心理课(段大夫、马大夫) 17层活动室	沙龙 (马大夫) 17层活动室	心理课程〔段大夫、马大夫〕 17层活动室	爱与序位(聂巧乐) 17层活动室	
6:00-7:00		晩餐 1层餐厅	晚餐 1层餐厅	晚餐 1层餐厅	晩餐 1层餐厅	晚 <b>餐</b> 1层餐厅	
7:00-8:00		营养课 (刘大夫) 17层活动室	spa (20-36周自选) 17层spa间	自由活动	自由活动	结题沙龙 (马大夫)	
8:00-8:30		加餐	加餐	加餐	加餐	加餐	
8:30-9:30		休息/整理	休息/整理	休息/整理	休息/整理	休息/整理	
9:30		睡觉	睡觉	睡觉	睡觉	睡觉	

# The second phase:



course photo share

































# 3. Meals

include three meals in the morning, lunch and dinner, and two additional meals, which are customized by the nutritionist and chef of the center of mom, and the ration of vegetables, proteins and fruits. In addition, for allergies, gestational diabetes pregnant women do personalized.



### 4. Accommodation environment

1) accommodation: CENTRE OF MOM

(1/2/16/17, block C, wanhao center, 7 jianguomen south street, dongcheng district, Beijing)

3) room arrangement:



- rooms for pregnant families 1701, 1702, 1703, 1705, 1706
- activity room 1707 and 2nd floor (yoga class on 2nd floor)
- staff room 1707

Self - provided items: except the check-in supplies.

4) meal time:

Breakfast - 8:00 am/extra meal - 10:00 am/lunch - 12:00 PM/extra meal - 15:00 PM/dinner - 18:00 / extra meal in the evening - 20:00 including 3 meals (breakfast, lunch and dinner), provided to pregnant women and their families, extra meal only provided to pregnant women.

- (\* meals will be served on the morning of October 3rd) where: breakfast, lunch and dinner will be served in the restaurant on the 1st floor/extra food will be delivered to the room.
  - \* no food or drink is allowed in the room except those provided by the chef.
- 5) Spa introduction: pregnant women from 20 weeks to 36 weeks can enjoy the Spa for once. T The appointment time is 7 PM and it will take about 1 hour and 50 minutes.

# 5. Introduction to sample collection

Blood, urine, saliva, swab samples, body composition and heart rate variability were collected on the first day and the last day respectively. Stool samples were collected daily.

Blood and swab samples shall be collected by the nurse;

Urine, saliva and feces will be collected by the staff after sampling and introduction.

The body composition was tested by the outpatient department of nutrition of union medical college hospital.

Heart rate variability analysis was performed by outpatient testing in the department of psychology, Peking union medical college hospital

- 1) oral swab-swab tube
- 2) vaginal swab swab tube
- 3) metabolism gene, blood routine, glycosylated hemoglobin blood purple tube
- 4) glycosylated albumin, FBG, HsCRP, triglyceride, liver function serum yellow head long tube
  - 5) insulin, c-peptide, proinsulin, interleukin 6, 8, 10 blood red head and yellow ring
  - 6) antibiotic detection -- blood red circle and black tube
  - 7) urine ACR+PCR, iso-pge2-white head catheter 4ml 8) urine rt-blue head catheter 10ml
  - 9) feces storage liquid tube in the stool box
  - 10) saliva white head tube 4ml

*号	生物样本	項目	数量	人数	实际检测数 量	采样盒	采样要求	特殊处理	转延方式	报告周期	储存地点
	口放拭子	16s RNA	2 (共尾)	5	10	口放拭子	-	冻存	护士	40天	检验科
	阴道拭子	16s RNA	2 (共尾)	5	10	阴道拭子	-	冻存	护士	40天	检验料
		代謝越四	2 (头尾)	5	10	族管	-	离心、冻存	护士	15天	检验料
		主体规	2 (英尾)	5	10	業管	-				
		糖化血红蛋白	2 (头尾)	5	10	敦莹	-				
		糖化白蛋白	2 (头尾)	5	10		-				
		纤维蛋白原 (FBG)	2 (共尾)	5	10		-				
	da.	HsCRP	2 (共尾)	5	10	黄头长管 血清管	-				
	-	車購三項(FFANLDC、TG、TC)	2 (头尾)	5	10		-				
		肝功 (ALT、Cr、Alb)	2 (头尾)	5	10		-	门途临榜	护士	1-4周	检验科
		題高家	2 (头尾)	5	10	紅头黄團	-				
		clk	2 (共尾)	5	10	紅大英國	-				
		胰岛黄原	2 (共尾)	5	10	红头黄圈	-				
	100	白介素6、8、10	2 (头尾)	5	10	紅头黄檀	-				
		PCR+ACR	2 (头尾)	5	10	白头尿管 4ml	-	7			
	尿	RT	2 (头尾)	5	10	蓋头尿管 10ml	-				
		ISO-PGE2	2 (头尾)	5	10	自头尿管 4ml (特殊5	-	中段、立即冻存	护士	50天	检验料
	美便 (每天图取)	16s RNA	2 (全程)	5	25 (美尾先锋	保存液管	-	常温/痣存	护士	40天	检验科
	<b>福液</b>	蛋白组学	2 (头尾)	5	10	白头管 4ml	-	冻存	护士	40天	检验科
	£1	拉生養給測	2 (头尾)	5	10	紅管原標	-	冻存	护士	10天	松田科

	两次抽血、五次口 5天6晚、5组家庭/	腔拭子、皮肤拭子、尿液、大便 \住						10月16日早空康 10.16早上寓开			
÷	生物样本	項目	数量	J.	数 实际检测数量	录样金	系样要求	特殊处理	转弦方式	报告周期	储存地点
П	口腔拭子	16c RNA	5 (全年	1 5	10 (気尾先催检測)	口節拭子	-	冻存	护士	40天	检验科
Т	別道杖子	16s RNA	2 (头角	5 18	10	防衛松子	-	本存	<b>护士</b>	40天	检验科
		代謝基因	2 (失声	5 5	10	常管	-	意心、 亦存	护士	15天	检验科
_	1	血管規	2 (美月	5	30	常管					
	1	<b>特化</b> 血红蛋白	2 (美角	5 18	30	常管	-	7			
	1	<b>特化白蛋白</b>	2 (54,8	šl 5	10		-	7			
	1	纤维:蛋白原 [FBG]	2 (美角	5 13	10	7	-	7			
	m.	HsCRP	2 (美角	5	10	黄头长管 血清管	-	1			
	1	血脂三项 (FFANLDC, TG, TC)	2 (美角	5	10		-	1			
Ď	1	SFID (ALT, Cr. Alb)	2 (美海	El 5	10		-	门诊療检	护士	1-4周	检验科
1	1	胰岛療	2 (美春	5 5	10	红头黄圈					
2	1	clk	2 (5,8	5	10	紅矢房間	-	7			
3	1	胰岛家族	2 (吳月	5 5	10	红头黄圈	-	7			
4	1	白介寮6、8、10	2 (吳声	5 5	10	红头黄圈	-	1			
5		PCR+ACR	2 (3.8	5 5	10	白.吳原管 4ml	-				
В	展	RT	2 (头角	5 18	10	蓝头尿管 10ml	-				
7		ISO-PGE2	5 (全者	5	10 (矢尾失傷检測)	白矢乐管 4ml (特殊分集)		中段、立切亦存	护士	500C	检验科
В	養便 (毎天開取)	16s RNA	5 (全年	5	10 (矢尾失俗检测)	保存液管		常温/亦存	护士	40X	检验科
9	環液	蛋白甜学	2 (头角	5 5	30	白头管 4ml		<b>本</b> 存	护士	40天	检验科
0	血	抗生素检测	2 (54.8	5 13	10	红管黑图	-	亦存	护士	10天	检验科
1	皮肤状子	共創样	5 (全意	5	只管样不能检测	拔子	-	亦行	护士	-	检验科

$ \begin{array}{cccccccccccccccccccccccccccccccccccc$												心丰变异性	分析										
	姓名	HERR	SDWN	EMS-SD	PNN50	TP	TLF	1.F	HF	LF/EF	心理波松滑数	身体放松物数	HRUT	SDOW	BMS-SD	PNN50	TP	TLF	LF	HF	1F/HF	心理放松指数	身体放松指数
	张神经		99	135	33.6	1113.2	100.96	63, 11	36, 89	1, 71	46		90.8	53	54	23, 44	735, 58	368, 72	26, 27	73, 73	0,36	32	- 64
	<b>#</b> ++	88.5	52	61	19, 32	758, 16	543, 97	61.86	38.14	1.63	38	25	93. 2	53	60	9,48	592, 42	482, 73	60.38	39, 43	1,52	39	49
	全非	92.7	90	103	10.24	3048, 74	1905, 34	29, 25	70.75	0.41	23		85.7	66	48	7.87	1944.86	1805. 5	39.99	60.01	0.67	20	31
	Here	87.4	- 66	32	9, 49	826, 78	586, 09	62, 18	37.82	1.64	28	1	112.9	15	10	1.07	108, 21	89.81	67.94	32, 46	2.12	28	56
\$\overline{\ov	<b>金**</b>		31	39	15, 57	192, 46	51. 67	45.4	54.6	0.83	32	42	105, 8	93	136		3477, 64	641.21	50, 53		1,02		25
\$\hat{k}\ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \	2]++	78.6	85	122	38, 26	681.59	373, 26	31.83	68.17	0.47	19	28	64.5	82	92	56,39	1916.97	221	25.11	74.89	0.34	55	- 1
Re+ 70.9 34 41 11.38 394.5 240.49 56.27 43.73 1.29 28 18 73 125 173 48.67 4195.22 622.98 31.52 68.48 0.46 19	张**	81.8	170	234	45, 43	5772, 56	1989, 74	13, 88	86.12	0.16	20	26	92.2	53	79	15, 51	358, 11	40.06	34.78	65, 22	0.53	19	18
	2]**	87	34	20	1,85	361, 33	131. 78	92.2	7.8	11. 82	63	34	84.6	53	.59	8,31	692, 62	225	89.84	10.16	8,84	62	23
## 87 2 76 89 27 04 1031 82 239 64 39 22 60 28 0 66 34 1 98 6 50 44 5 93 707 29 485 75 66 89 33 11 2 02 38	<b>R</b> **	70.9	34	41	11,33	394.5	240, 49	56, 27	43, 73	1, 29	28	18	73	125	173	48, 67	4190, 22	622, 98	31.52	68, 48	0,46	19	
	拼响	87.2	70	88	27.04	1033, 82	239, 64	39.72	60.28	0.66	34	1	98. 6	50	44	5.93	707.29	485.75	66.89	33, 11	2.02	38	

			12	成分				
姓名	Body weight/kg	Body Weight/kg	Muscle content/kg	Muscle content/kg	Fat rate/%	Fate rate/%	水分前	水分后
庚**	66.8	65.2	21.6	20.8	38	39.5	0.398	0.39
金**	71.3	70.5	24.4	24.5	36.3	35.7	0.39	0.38
高*	75.1	75.7	25.6	26.9	37. 2	35	0.388	0.38
刘**	62.2	61.4	23.7	23.2	29.4	30.3	0.387	0.38
刘**	54.4	53.9	21.3	20.6	27. 1	28.8	0.383	0.38
金**	49.1	49.6	16.7	16.7	34.3	35.3	0.39	0.38
<b>秦**</b>	57.3	56.7	21.3	21.1	31. 2	31.4	0.38	0.37
张**	55.9	56.5	22.9	23.2	23.4	23	0.395	0.39
蔡**	76.5	76.6	30.8	30.4	27. 1	27.8	0.382	0.38
张**	61.8	62.1	25.4	25.1	24.6	25.6	0.383	0.38
Mean	63. 04	62. 86	23.37	23. 25	30. 86	31.24	0.3876	0.386
	5 increase;	5 decrease	7 decrease,	3 increase	3 decrease	, 7 increase	8降2升变化	化不大

# 6.Maternity feedback

There are barriers to any career and training is required, but parenting lacks training. The five-day perfect pregnant moms' camp is a training camp for expectant fathers and mothers. The five-day course provides you with the opportunity to learn how to produce and how to face gestation, and you also have a more systematic and in-depth understanding of health management. Through close communication with the most professional doctors, I have interpreted many misunderstandings during pregnancy and learned safer and more effective ways of exercise during pregnancy. The five days spent together between husband and wife have made the relationship between husband and wife more harmonious and harmonious, eased the tension and anxiety of being a mother and a father for the first time, treated the birth more relaxed and wonderful, and welcomed the birth of a new life with full confidence.







7. Outcome of childbirth

10 people have participated in the project and 10 have given birth. Five cases were born naturally at full term. Two patients underwent cesarean section at the buttock position, and one patient underwent cesarean section at the occipital posterior position due to continuity. One case of postoperative hypothyroidism of thyroid cancer was less than gestational age.

A case of premature delivery at 30+5 weeks of gestation, neonatal asphyxia, combined with uterine fibroids, uterine fibroids removed after surgery;

- 1) Kim \*\*, 2019.03.11 delivery, 38 weeks +4, natural delivery
- 2) liu \*\*, 2019.02.26 delivery, 37 weeks +5, natural delivery
- 3) zhang \*\*, 2019.03.11 delivery, 37 weeks +6, cesarean section
- 4) zhang \* ting, 2018.12.18 delivery, 41 weeks +1, cesarean section
- 5) CAI \*\*, 2018.12.12 delivery, 38 weeks +3, natural delivery
- 6) geng \*\*, 2019.03.16 weeks before birth, 39 weeks before natural birth
- 7) gao \*\*, 2018.10.28 delivery, 30 weeks +5, cesarean section
- 8) liu \*\*, 2019.1.5 labor, 39 weeks +2, cesarean section
- 9) qi \*\*, 2019.1.15 delivery, 39 weeks +6, natural delivery
- 10) jin \*\*, 2018.12.8 delivery, 39 weeks +6, natural delivery

10月完美孕妈										
课题编号	分娩时间	分娩孕周	分娩方式(自然分娩)	分娩方式(制官产)	新生儿性别-男	新生儿性别-女	新生儿身长(co)	新生儿体重{g}	阿氏评分-1分钟	阿氏评分-5分钟
1	2019.03, 11 14:44	3814	1			1		3520	10	10
2	2019.02.26	37=5	1			1	48	2240	10	10
3	2019.03.06 10:55	37+6		1		1		3330	10	10
4	2018.12, 18 20:06	41+1		1	1		50	3590	10	10
5	2018.12,12 19:42	38+3	1			1	48	3200	10	10
6	2019.03.16	39	1			1	49	3045	10	10
7	2018.10.28 20:22	30+5			1		38	1120	7	10
8	2019.1.5 06:33	39+2		1		1	53	3910	10	10
}	2019. 1. 15	39+6	1			1	49	3225	10	10
10	2018.12.08 19:08	39+6	1		1		51	8240	10	10

- $\equiv$ 、Clinical test analysis report
- 1. There was no significant difference in body weight and body composition before and after 5 days

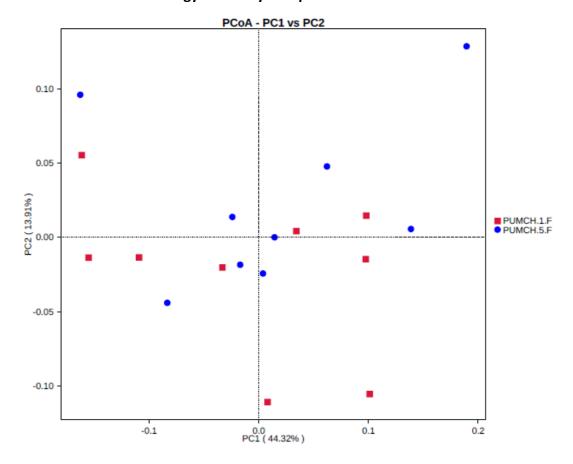
P	relimi	nary r	esult	-///	and our mint	和脊院
Y		В	ody compo	sition		
	Body weight/kg	Body Weight/kg	Muscle content/kg	Muscle content/kg	Fat rate/%	Fate rate/%
#	66, 8	65, 2	21.6	20.8	38	39.5
接金度対対金素张裝张	71.3	70.5	24.4	24.5	36.3	35.7
*	75.1	75.7	25.6	26.9	37.2	35
26	62.2	61.4	23.7	23, 2	29.4	30.3
Ħ	54.4	53.9	21.3	20.6	27,1	28.8
ŝ	49.1	49.6	16.7	16.7	34, 3	35.3
素	57.3	56.7	21.3	21.1	31.2	31.4
张	55.9	56.5	22.9	23. 2	23.4	25
栗	76.5	76.6	30.8	30.4	27.1	27.8
96	61.8	62.1	25.4	25.1	24. 6	25. 6
Zeun	63.04	62.85	23, 37	23, 25	30, 85	31.24
	5 increase	5 decrease	7 decrease.	3 increase	3 decrease	7 increase

# 2. Metabolic and assay indexes test

There was no significant difference before and after five days

10月完美孕妇	4					
课题编号	Total cl	nolesterol	Triglyce	eride	LDL	
1	4.25	4.24	1.35	1.17	2.32	2.25
2	5.68	5.78	1.52	1.94	3.08	2.92
3	5.19	4.85	4.73	2.63	1.57	1.58
4	5.95	6.09	1.84	1.91	3.05	3.1
5	5.02	4.78	2.62	2.98	2.13	2.04
6	6.63	6.41	1.35	1.18	3.67	3.7
7	5.78	5.25	2.84	2.39	2.97	2.67
8	8.53	8.49	3.01	3.18	4.86	4.97
9	6.39	6.37	2.06	2.15	2.92	2.94
10	5.75	6.13	2.09	3.05	3.1	3.05

# $\Xi_{\mathsf{v}}$ Intestinal microecology 16s analysis report



# 四、Report of saliva metabolomics experiment

# 1. Quality analysis

data\_processed-P-0.05.M5 (PCA-X) Colored according to classes in M5

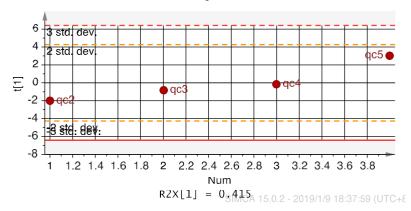


图 1 质控样本偏差分析 质控样本稳定性较好,本批数

据符合分析要求

# 2 PCA Clustering analysis

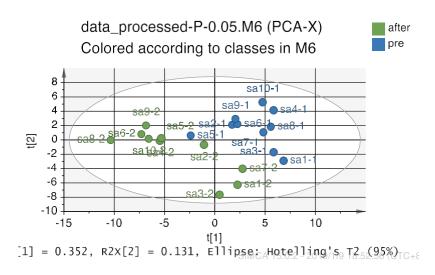


FIG. 2 PCA Clustering analysis

As shown in FIG. 2, the two groups showed obvious classification trend, indicating that there were certain differences in the metabolome.

### 3 OPLS-DA anaysis

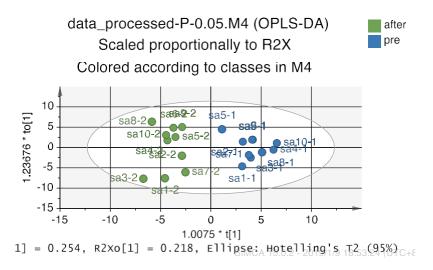


Fig 3 OPLSDA

# analysis

Further supervised clustering analysis showed that the difference between the two groups was more significant, as shown in figure 3

According to the OPLS-DA model, pathway analysis of the different variables was carried out, and the results showed that the metabolic pathways significantly changed in the intervention group were sialic acid metabolism, vitamin C metabolism, aldose metabolism, pyrimidine metabolism, and hexosan phosphorylation (as shown in figure 4).

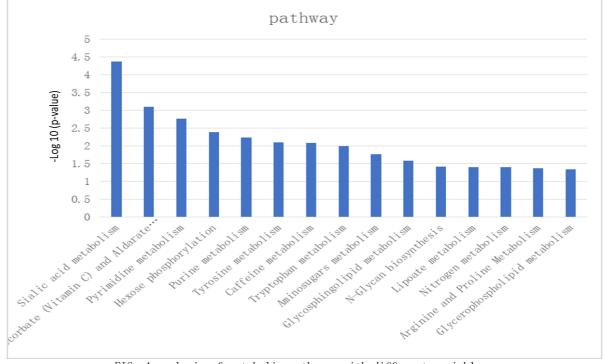


FIG. 4 analysis of metabolic pathways with different variables

# $\pm$ . Non-targeted metabolomics experiment report

# 1. Sample information

本实验共收集样本 20 个人血清,分为 2 组,Before 组、After 组,每组分别有 10 个样本,样本详细信息见表 1。

样本名称	分组
1-1	Before
2-1	Before
3-1	Before
4-1	Before
5-1	Before
6-1	Before
7-1	Before
8-1	Before
9-1	Before
10-1	Before
1-2	After
2-2	After
3-2	After
4-2	After
5-2	After
6-2	After
7-2	After
8-2	After
9-2	After
10-2	After

表 1 样本信息 经实验员核

验,收到的样本与样本信息一致,保存于-80℃,直至正式开展实验。 2. 实验信息

# 2.1 Instruments and reagents

# 2.1.1 试剂

试剂	CAS	纯度	品牌
水	7732-18-5	LC-MS	Thermo
乙腈	75-05-8	LC-MS	Thermo
甲酸	A117-50	LC-MS	Thermo

表 2 试剂

# 2.1.2 仪器

仪器	型号	品牌
超高效液相	Waters ACQUITY UPLC I-Class	Waters
高分辨率质谱	Waters XevoG2-XS Qtof	Waters
色谱柱	Waters HSS T3 column	Waters
离心机	Legend Micro 17R	Thermo

#### 2.2.1 代谢物提取

- 1. 100 uL 血浆/血清+300 uL ACN (-20℃ 预冷), 涡旋振荡 1 min 后, 于-20℃静置过夜;
- 2. 1,2000rpm, 4℃离心 20 min,取上清,1×水稀释,取其中 100ul 到进样瓶;
- 3. 各取 50ul 制作 QC 样本。

2.2.2 仪器参数

柱温 (℃): 45

样品温度 (℃): 4 液 相流速: 0.45ml/min A 相: 水 +0.1%甲酸

B 相: 乙腈+0.1%甲酸

时间	A%	В%	Curve
initial	95	5	initial
1.5	80	20	6
15. 0	10	90	6
18.0	0	100	6
18. 1	95	5	6
20.0	95	5	6

表 4 液相条件

#### 2. 质谱条件

在正离子采集模式下,Masslynx 软件基于 MS<sup>E</sup> 功能对样品进行一级、二级质谱数据采集。毛细管电压:2.5kV,锥孔电压 24V,离子源温度 100 ℃,去溶剂气流速 800 L/h,锥孔气流速 50 L/h,14min 内对 m/z 为 50-1500Da 的离子进行扫描,0.2sec/ 循环。

在负离子采集模式下,Masslynx 软件基于  $MS^{\epsilon}$  功能对样品进行一级、二级质谱数据采集。毛细管电压:2.5kV,锥孔电压 25 V,离子源温度 100 °C,去溶剂气流速 600 L/h,锥孔气流速 10 L/h,14min 内对 m/z 为 50-1500Da 的离子进行扫描,0.2sec/循环。

# 2.2.3 上机检测

为了更好地采集数据,确保仪器的最佳状态,在正式样品上机前需要用 QC 样本做稳定性测试,一般将同一个 QC 样本重复进样 5 针左右,此样本为 condition QC,待仪器稳定方可进样。每 10 针样本中间插入一个 QC,确保仪器进样过程中的稳定性。

# 3 The experimental results

#### 3.1 data results

在本次实验中负离子模式共检出了 7688 个 features,正离子模式共检出了 8609 个 features。其中 QC 样本的 BPI (base peak intensity,基峰图)如下图所示,纵轴表示峰强度,横轴表示出峰时间,左上角标明样品名称,右上角表示 intensity。BPI 图中的峰型比较窄、数量多,表明色谱分离效果好。

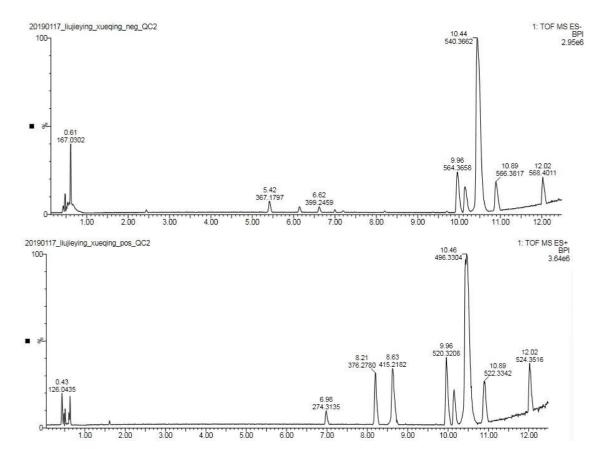


图 1 QC 样本负离子(ES-)模式和正离子模式(ES+)BPI图

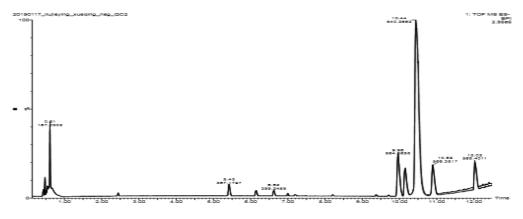
位置: LKM-产科-非靶向代谢组-20-血清-20190101\\峰图信息

# 3.2 Data quality control

色谱质谱联用仪器构造精密,在使用过程中有很多因素会造成样品采集的系统误差,比如:温度、湿度、仪器的清洁程度等。所以,保证仪器稳定性是实验成功能的基础。我们在两个方面做好质控保障:

### 1. 过程质控

在仪器进行数据采集的过程中,我们将插在样品中间的 QC 样品做 overlay,确定保留时间和峰强度基本保持不变,详见 2。纵轴表示峰强度,横轴表示出峰时间,左上角标明样品名称,每个颜色的线条表示一个样本的出峰情况,overlay 越好,表明样本出峰的一致性越好。



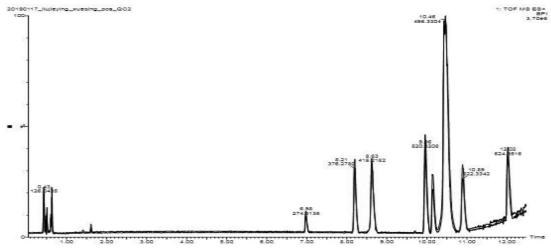


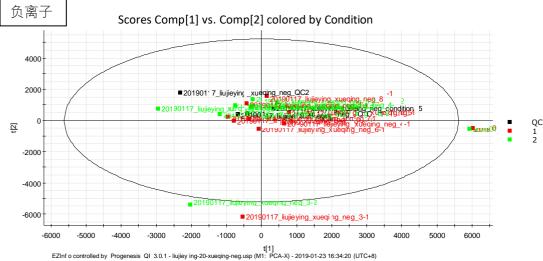
图 2 负离子 (ES-) 模式和正离子(ES+)穿插在样本中的 QC BPI overlay 图

位置: LKM-产科-非靶向代谢组-20-血清-20190101\\峰图信息

# 2. Data quality control

#### a. QC 样本在 PCA 分析中的表现

样本 PCA 分析,主要是对数据进行降维分析,可以检测实验组间的差异性及组内的重复性。PCA 分析计算有关代谢物组 成的主成分,在二维图中,我们取前两个主成分 PC1, PC2 来表示样本,空间分布差异越小,表示两个样本的数据越接 近。图中每个点代表一个实验样本,并以不同颜色区分不同分组。当仪器稳定时,QC 样本相对集中在一起,不存在随时 间变动的情况。



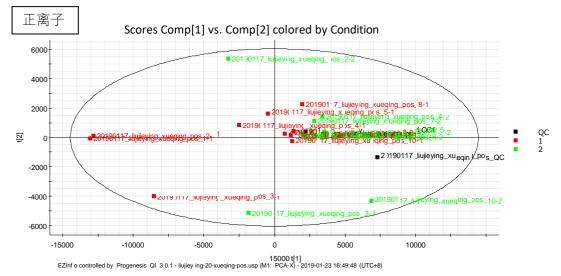


图 3 PCA分析,本图是通过 Waters QI 软件对代谢组数据进行分析获得的,QC 样本分布比较集中,仪器重复性好位置:LKM-产科-非靶向代谢组-20-血清-20190101\\非靶向代谢组##离子模式\\ presentation-##

b. QC 样本的相关性 为考察研究样本多次生物学实验的重复性,基于组间实验共定量小分子的强度值进行相关性分析,散点分布图如图

4 所示,横坐标和纵坐标分别为该组实验样品代谢物强度值取 log2,任意两组重复实验共定量多肽强度值的 spearman 相关系数如图上标注所示。相关性分析所涉及的数据以及图均在相应离子模式数据中的 correlation 文件夹中。

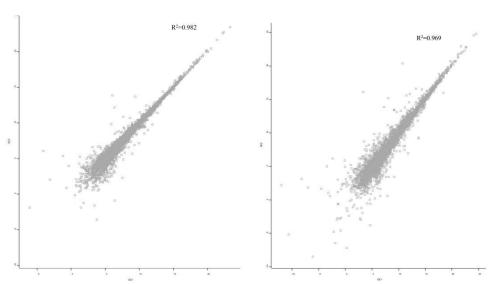


图 4 QC 样本相关性分析,图中数据的相关系数均大于 0.80,显示了极好的一致性,左图为负离子模式,右图为正离子模式

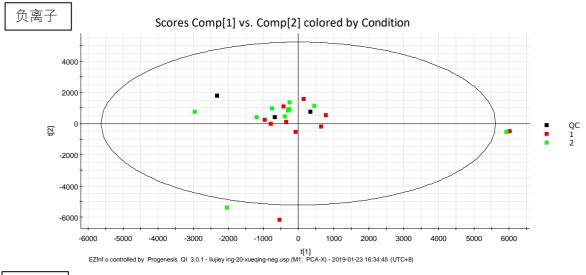
位置: LKM-产科-非靶向代谢组-20-血清-20190101\\非靶向代谢组##离子模式\\ QC 样本相关性分析 4 数据分析

# 4.1 Basic data analysis

# 4.1.1 PCA analysis

获得原始数据后导入 Progenesis QI(Waters)软件进行数据预处理、对齐、去卷积、峰提取、多元统计分析和鉴定。将原始数据导入后,选择自动模式进行数据处理,通过 Lockmass 进行质量分数校正,QC 样本作为参照组进行峰对齐。通过 EZinfo 软件内置统计分析方法筛选差异代谢物。PCA 分析是 EZinfo 软件分析的第一步,主要是对数据进行降维分析,可以检测实验组间的差异性及组内的重复性。在二维图中,取前两个主成分 PC1,PC2 来表示样本,空间分布差异越小,表示两个样本的数据越接近。图中每个点代表一个实验样本,并以不同颜色区分不同分组。重复性较好的实

验,同一组内的不同样本应该聚集在一个相对集中的范围内,并可以与其他组的数据聚集区域区分开。



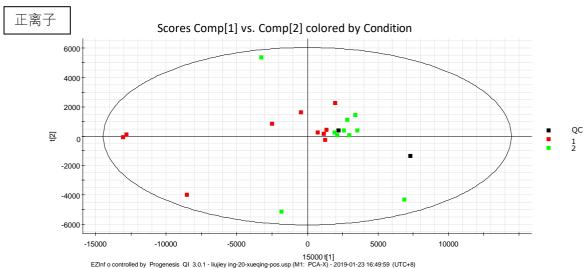


图 5 PCA 分析,图中三种颜色分别代表三种样本分组,每个圆形代表这一个样本的代谢组降维处理后投射在二维平面上的位置,横纵坐标上的百分数表示组间在这一方向的差异中可以解释全面分析结果的百分比,百分比越大表示在这一方向上的区分度越好。

位置: LKM-产科-非靶向代谢组-20-血清-20190101\\非靶向代谢组##离子模式\\ presentation-## 4.1.2 OPLS-DA(正交偏最小二乘法-判别分析)

为了消除与分类不相关的噪音信息,同时也为了筛选导致分类差异的可信代谢物,选取 OPLS-DA 分析过滤与分类不相关的信号,即正交信号,获得 OPLS-DA 模型,对模型的质量用交叉验证法进行检验(即用一部分样本数据制作分组模型,另外一部分数据用来测试已分组的模型),得到的 R2X 和 Q2 分别代表模型可解释的变量和可预测度,可对模型的优劣进行判别,原则上,R2X 和 Q2 越接近 1,模型越好,同时 R2X 和 Q2 的差值最好不大于 0.2(详见图 6 和 PPT 中数据分组情况)。通过模型分析可以对代谢物进行 VIP 打分筛选,VIP 分数越高的代谢物,对分组的贡献越大,本实验选取在QC 样本中 CV 值小于 30%的代谢物、VIP>1、Pvalue<0.05 的代谢物为差异代谢物进行搜库,database 为 HMDB、KEGG 和 NIST 数据库,差异代谢物列表详见《before vs after 差异—##. xlsx》文件。表中的代谢物均为此两组比较的差异代谢物,代谢物信息和相关文献请见列表最后一列的网站链接,或通过 "Accepted Compound ID" 和 "Accepted Description"在 pubmed 上进行文献查找; "Normalised abundance"列为代谢物归一化后的定量值列,后续数据分析均通过归一化后的定量值计算获得, "Anova(p)"和 "Max Fold Change"列为计算的差异代谢物的显著性和变化倍数,可通过这两列 挑选变化最显著的差异代谢物。 "Highest Mean"和 "Lowest Mean"分别表示高表达量组和低表达量组。《all compound measure.csv》为所有鉴定代谢物定量列表,《change compound iden.csv》为所有差异代谢物鉴定可能性列表(由于非

靶向实验没有标准品比对,所有代谢物鉴定通过"Score(代谢物鉴定总打分)"、"Fragmentation Score(二级谱打分)"、"Mass Error (ppm)(质量偏差)"和"Isotope Similarity(同位素峰分布打分)"综合打分进行挑选最佳匹配的代谢物命名,鉴定可能性列表包括所有差异的 features 对应的代谢物鉴定可能性信息)。

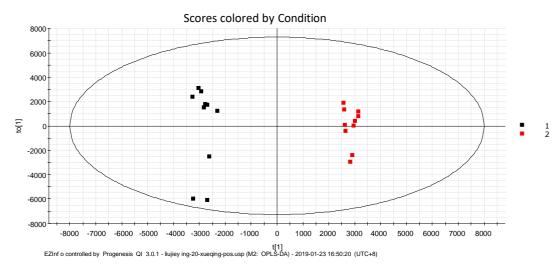


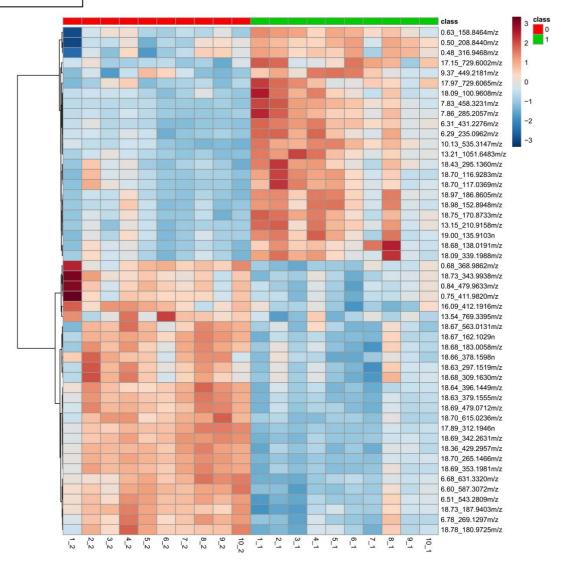
图 6 OPLS-DA 分析,图中颜色分别代表样本分组,每个图形代表这一个样本的代谢组降维处理后投射在二维平面上的位置,此实验中的OPLS-DA 模型的 R2Y 和 Q2 分别是 99%和 90%,数据显示模型不存过拟合的现象,模型对分组有预测能力(其他 OPLS-DA 图,详见对应离子模式的 PPT 中)。

位置: LKM-产科-非靶向代谢组-20-血清-20190101\\非靶向代谢组#离子模式\\ presentation-## 4.2

### Personalized data analysis

### 4.2.1 Hierarchical clustering analysis

clustering analysis is a common method in pattern recognition and data mining. It is an effective method of knowledge discovery based on data. Where, in the vertical axis, the metabolites are clustered, and the depth of color is used to represent the amount, and the horizontal axis is the sample information. Based on the screened differential metabolites in the samples, the union set of differential metabolites was taken for hierarchical clustering, and the results were shown in FIG. 7.



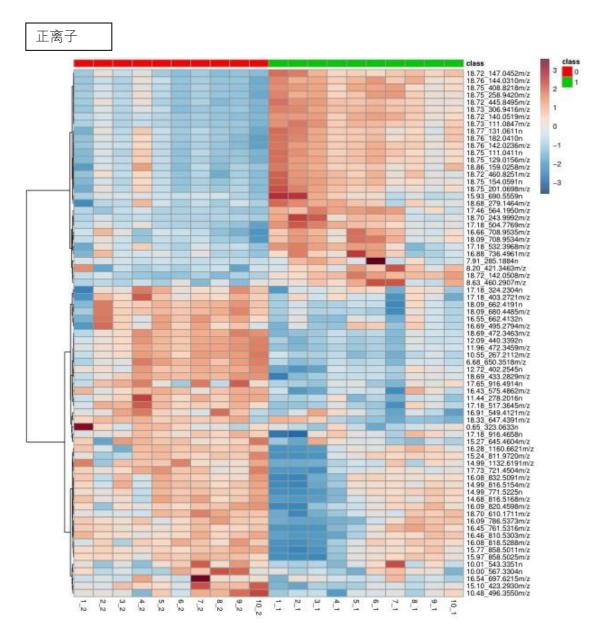


Fig. 7 analysis diagram of the aggregation hierarchical clustering of cation differential metabolites. The abscissa represents the sample name and sample classification, and the ordinate represents the clustering result of differential metabolites. Color represents the peak strength of metabolites, red represents high expression, and green represents low expression. Sort according to pvalue, and take the first 50 metabolites for drawing.

Item: LKM- obstetrics - non-target metabolome -20- serum -20190101\\ non-target metabolome ## ion model \ hierarchical clustering analysis 4.2.2 analysis of metabolic pathway

According to the statistical analysis, the compound name of different metabolites in each set of positive and negative ion models was input into IMPaLA. The hypergeometric test algorithm was selected and the different metabolites were compared with the database to obtain the list of different metabolites participating in the pathway (see attached table "pathway analysis"). The data is represented by a histogram.

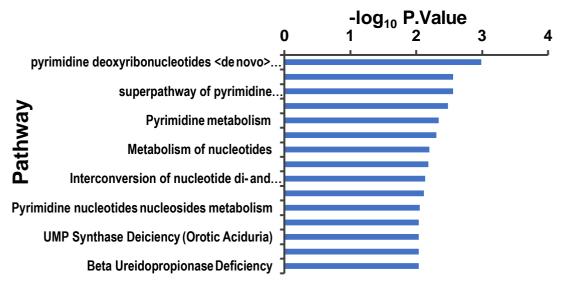


FIG. 9 pathway analysis diagram

In the figure, the horizontal axis is -log10 Pvalue, the smaller the value is, the more reliable the enrichment significance of differentially expressed metabolites in this pathway is, and the horizontal axis is the enriched metabolic pathway.

Location: LKM- obstetrics - non-targeted metabolome -20- serum -20190101\ pathway analysis 5 Reference

- [1] Jové M, Ayala V, Ramírez-Núñez O, et al. Lipidome and metabolome analyses reveal potential plasma biomarkers of early atheroma plaque formation in hamsters[J]. Cardiovascular Research, 2012, 97(4):642-652.
  [2] Winder C L, Dunn W B, Schuler S, et al. Global metabolic profiling of Escherichia coli cultures: an evaluation of methods for quenching and extraction of intracellular metabolites. [J]. Analytical Chemistry, 2008, 80(8):2939-48.
- [3] Antonio V P, Tuulikki S L, Gema M G, et al. Bioinformatics strategies for lipidomics analysis: characterization of obesity related hepatic steatosis[J]. Bmc Systems Biology, 2007, 1(1):1-15.
- [4] Mcphail M J W, Shawcross D L, Lewis M R, et al. Multivariate metabotyping of plasma predicts survival in patients with decompensated cirrhosis:[J]. Journal of Hepatology, 2016, 64(5):1058.

# 六、Summary and discussion

In this perfect pregnant mothers training camp, there was no difference in clinical test and microbiome between the 10 pregnant mothers before and after participating in health management. Metabolomics analysis found that, Metabolic pathways that changed significantly after intervention were sialic acid metabolism, vitamin C metabolism, uronic acid metabolism, pyrimidine metabolism, hexoses phosphorylation, and non-targeted metabolomics analysis found that differentiated metabolites were involved in pathway changes.

Ten pregnant mothers had excellent experience and feedback on the perfect pregnant mother training camp, and the delivery outcome was good.

1. Direction exploration: gold samples were obtained through centralized management of healthy pregnant women from diet, sports, psychology, health education, accommodation environment, family education (inviting family members to participate), etc., so as to find the direction for the establishment of gold sample database. The acquisition of gold samples, for example, provides good evidence for new treatment regimens, clinical interventions and non-drug level interventions, which is like a graduated ruler to set standards. It is also the biggest goal of this research direction.

### 2. Transformation mode exploration:

# 1) Business transformation:

Through this project, this centralized training camp could be transformed into products, which could be delivered to high-level maternal and infant education, high-level maternity center, high-level private hospitals and other places, forming a business model and industrial chain. We can also discuss with the insurance company to form the service project of high-level health management insurance.

### 2) scientific research transformation:

the sample size of this research is too small to be stratified, the research time is short and the samples are all from Beijing area, which are biased to some extent. The intervention plan is very detailed, but lacks some quantitative indicators and more rigorous scientific theoretical guidance (study design, outcome tracking). The participation and compliance of pregnant women are very satisfactory and highly recommended by the subjects. However, if the sample size is enlarged, it may cause some difficulties in the intervention and implementation. However, the establishment of the gold sample database is a very valuable direction. If 50 or even 100 cases of such a model are implemented, more data will become effective support. At the same time, we can also compare the healthy biological samples with diseases during pregnancy to draw more effective conclusions.

# 3) community, the family

promote this model to the community, so that the pregnant women and family can accept the effective management without leaving home, the implementation can use short-term learning as the standard, and when returned home after studying, according to the requirements, they could have a management for themselves and family, in order to improve the obstetric risk awareness, reduce the occurrence of complications (as the core purpose). We will also continue to obtain information and follow up of the subjects to collect more complete data and more analysis for this project.

Thanks again for the great support from Hanmi!

Thanks for the help from Center of Mom!

Thanks to the clinical medical staff, basic scientific research staff, service staff and scientific research assistants who participated in the completion of the project!

Thank you for your active participation and feedback!