Women's age and total motile normal morphology sperm count predict fecundability: a prospective cohort study

Mei Ting Lam[1,2];

Hang Wun Raymond Li[1,2,3];

Ching Yin Grace Wong[1,3];

William Shu Biu Yeung[1];

Pak Chung Ho[1];

Ernest Hung Yu Ng[1]

[1]Department of Obstetrics and Gynaecology, The University of Hong Kong, Queen Mary Hospital, Pokfulam Road, Hong Kong

[2]Department of Obstetrics and Gynaecology, Kwong Wah Hospital, Hong Kong

[3]The Family Planning Association of Hong Kong

Corresponding author: Dr. MT Lam

Address: North Wing, 10th Floor, Kwong Wah Hospital, 25 Waterloo Road, Kowloon, Hong Kong

Telephone: +852-35175474

Fax: +852-35177149

Email: lmt256@ha.org.hk

Abstract

Objective(s): This study investigated the role of women's age, serum anti-Mullerian hormone (AMH) level and semen parameters in predicting fecundability.

Design & Methods: This was a prospective cohort study on couples attending for preconceptional health check. Occurrence of conception at one year after ceasing contraception and time to pregnancy were noted by phone follow-up. The women's age, serum AMH level, and total motile normal morphology sperm count (TNMC), were compared between those who conceived and not after one year; their independent predictive value on conception at one year was analysed by logistic regression. Among those conceiving within one year, Spearman's correlations between time-to-pregnancy and the clinical parameters were studied.

Results: Of the 100 couples analysed, we found younger age of the women (P=0.008), higher serum AMH level (P=0.038) and higher total motile normal morphology sperm count (TMNC) (P=0.015) in those conceived within one year. Multivariate logistic regression found that women's age (odds ratio 0.867, 95% CI 0.761-0.988, P = 0.032) and TMNC (odds ratio 1.089, 95% 1.001-1.185, P = 0.047), but not serum AMH level, significantly predicted conception within one year. Among those conceived within one year, none of the parameters analysed were correlated with time to pregnancy within one year.

Conclusions: Women's age and total motile normal morphology sperm count are significant independent predictors of conception within one year. No parameter was shown to predict

the time to pregnancy within one year. This finding can aid preconceptional counselling of couples who are planning for pregnancy.

Key message points

1. Women's age and total motile normal morphology sperm count, but not AMH level, were significant independent predictors of conception within one year.
2. Lower HDL-cholesterol levels in the female and male were found in couples conceived within one year, but the magnitude of difference was very small.
3. In our cohort, couples conceiving within one year or not showed no differences in men's age, body mass index, other lipid parameters, physical activity and stress score.

Introduction

Fecundability is the probability of a pregnancy, during a single menstrual cycle in a woman with adequate exposure to sperm and no contraception, culminating in a live birth.[1] Time to pregnancy is the time taken to establish a pregnancy, measured in months or in numbers of menstrual cycles.[1] There is a trend towards delaying childbearing resulting in more women facing fertility problem at the time they hope to conceive.[2] If the factors affecting fertility could be identified, it might be possible to offer counselling on family planning and possible early fertility intervention. Various factors have been studied for their influence or predictive value on fecundability, particularly those related to the production and competence of gametes, such as women's age and serum AMH level as well as semen parameters.

Anti-Mullerian hormone (AMH) is a polypeptide hormone solely secreted by granulosa cells of preantral and small antral ovarian follicles in the adult female. Serum AMH level demonstrates negligible fluctuation throughout the menstrual cycle,[3] and hence would represent a reliable marker of the ovarian reserve.[4] As ovarian aging is one important factor leading to reduced fecundability in women, studies have tried to explore the ability of AMH measurement to predict natural fecundability in the general population with contrasting results.[5-10]

On the other hand, although semen analysis is a standard investigation for couples presenting with subfertility, and that the 5th edition of the World Health Organisation (WHO) manual (WHO, 2010) revised the reference range based on a more evidence-based approach [11], its role in predicting fecundability in the general population still remains poorly defined [12] and it was not adopted in many studies looking into fecundability. [5, 8-10]

Besides, the correlation between a number of health and lifestyle factors such as coital frequency, obesity, metabolic problems and physical activity with fecundability is also of interest. Obesity as well as higher non-fasting serum free cholesterol concentrations in both men and women are associated with reduced fecundability.[13-15]

Other lifestyle factors may also impact on fecundability. It was reported that physical activity has influence on fecundability, in that physical activity of any type might improve fertility among overweight and obese women, whereas lean women who substitute vigorous exercise with moderate exercise may have improved fertility.[16] Perceived stress was also reported to be associated with reduced fecundability in women.[17]

Apparently, fecundability is subjected to influence by multiple factors in both the male and the female, and yet most of the existing studies were examining individual factors without taking into consideration the partner's factors. We carried out this prospective observational study to investigate the independent effects of age, serum AMH level and semen parameters, when controlled for each other, on fecundability in couples planning for conception. The effect of coital frequency, obesity, metabolic factors, stress level and physical activity were also studied. We hypothesized that these factors have potential effect on fecundability.

Methods

Subjects and data collection

This was a prospective observational study with one year follow-up. Participants were recruited at the Pre-pregnancy Check-Up Service of the Family Planning Association of Hong

Kong, a primary sexual and reproductive healthcare institution. Recruitment was conducted between November 2015 and April 2017, with the last follow-up completed in March 2019. They were couples who were planning to conceive, had stopped contraception for not more than six months or about to stop contraception, and the women were nulliparous and aged between 20 to 44 years. Exclusion criteria included history of infertility, having no sexual exposure or history of coital dysfunction, history of tuboperitoneal disease, pelvic inflammatory disease, ectopic pregnancy or endometriosis, anovulation or irregular menstrual cycles (cycle length <21 or >35 days), known endocrine disease, use of hormonal treatment which might affect ovarian or testicular functions within three months, use of injectable hormonal contraception within six months, men with azoospermia, and known medical or genetic diseases of the couple which might affect fertility.

Written informed consent were sought from the participants. The research nurse conducted interview with the couple to obtain demographic information, medical and reproductive history using a standardised questionnaire. The English and Chinese versions of the International Physical Activity Questionnaire – Short Form (IPAQ-SF)[18,19] were used to assess physical activity for all participants. It was expressed as Metabolic Equivalent Task (MET) - minutes per week. Stress level was measured by the Perceived Stress Scale score (PSS-10)[20]. Anthropometric measurements of the couple including body height and weight were recorded. Serum AMH level of the women was measured by the Access® AMH assay (Beckman-Coulter Inc., Marseille, France).[21] HbA1c and lipid profile were measured for the couple. Semen analysis was done according to the World Health Organization manual (5th edition). If there was abnormal parameter(s), another semen analysis was repeated three months later. The sample was collected after sexual abstinence for 2-7 days. The mean value was taken if the first one was abnormal and semen analysis was repeated.

Phone follow-up was conducted at six and 12 months after recruitment to obtain information on contraception, coital and pregnancy history. For couples who had regular unprotected intercourse for less than 12 months, phone follow-up was repeated after trying for 12 months. Couples who did not have regular unprotected intercourse 12 months after recruitment were withdrawn from the study.

Outcome measures

The primary outcome measures were conception at one year and time to pregnancy in those who conceived. Conception was defined as positive urine pregnancy test as reported by patients in telephone follow up. Time to pregnancy was counted from the time when the couple started to have regular unprotected intercourse without contraception.

Statistics

A minimum sample size of 81 would be adequate to determine a statistical significance between a Receiver Operating Characteristics (ROC) curve with area under the curve of 0.75 and one with the null hypothesis value of 0.5, with power of 80% and type I error of 0.05. Allowing for a drop-out rate of 30% which would be excluded from data analysis, we intended to recruit 120 couples. This would also fulfil the recommended minimum sample size of 100 for estimating the predictive value of individual factors on infertility in a univariate logistic regression model.

Continuous variables, expressed as median (25-75th percentile), were compared between those who conceived and not after ceasing contraception for one year by Mann-Whitney U test. Fisher's exact test was used to compare categorical variables between groups. Univariate binary logistic regression was used to study the predictive value of those individual parameters on conception at one year. Age and serum AMH level of the women, total motile normal morphology sperm count (TMNC) and coital frequency were entered into a standard multivariate binary logistic regression model to study their predictive roles after controlling for each other. ROC curve analysis was applied to determine the predictive value on conception at one year. Among those who conceived within one year, correlation between clinical parameters and time to pregnancy was determined by Spearman's correlation. Statistical analysis was performed using the IBM SPSS version 25.0 (IBM Corp, Armonk, NY, United States) and MedCalc Statistical Software version 15 (MedCalc Software bvba, Ostend, Belgium). A two-tailed value of $P < 0.05$ was considered statistically significant.

Patient and Public Involvement

This was solely an investigator-initiated study. There was no patient or public involvement in the design or execution of this study.

Results

A total of 112 couples were recruited, of which 100 completed the study and were eligible for analysis. Among the other 12, five were lost to follow up, five did not attempt conception by the end of the study period, one divorced and one had assisted reproductive treatment in another clinic before the end-of-study follow-up. For the 100 couples, all women were Chinese, 99 men were Chinese and one man was Caucasian. Two women were using hormonal contraception as their last method of contraception, one was using intrauterine device whereas the rest were using barrier or natural contraception. Characteristics of the participants are shown in Table 1.

Table 1: Comparison of basic demographic and clinical parameters between conception within one year or not. Categorical variables are presented as n (%) while continuous variables are presented as median (25^{th} – 75^{th} percentile).

		Overall	Conception within 1 year		P value
			yes (n=61)	**No (n=39)**	
Coital frequency at recruitment	>=2 per week	24 (24)	14 (23.0)	10 (25.6)	0.824
	Once per week	36 (36)	23 (37.7)	13 (33.3)	
	1-3 per month	35 (35)	20 (32.8)	15 (38.5)	
	<1 per month	5 (5)	4 (6.6)	1 (2.6)	
Coital frequency at last follow up	>=2 per week	41 (41)	27 (44.3)	14 (35.9)	0.240
	Once per week	36 (36)	18 (29.5)	18 (46.2)	
	1-3 per month	23 (23)	16 (26.2)	7 (17.9)	
	<1 per month	0 (0)	0 (0)	0 (0)	
FEMALE					
Age (years)		31.8 (29.7- 34.1)	31.1 (29.2-33.3)	33.1 (30.5-35.0)	0.008*
Education level	Secondary	12 (12)	6 (9.8)	6 (15.4)	0.530
	Tertiary or above	88 (88)	55 (90.2)	33 (84.6)	
Smoking	Never	96 (96)	59 (96.7)	37 (94.9)	0.731
	Quitted	3 (3)	1 (1.6)	2 (5.1)	
	Current	1 (1)	1 (1.6)	0 (0)	
Alcohol	Non-drinker	32 (32)	18 (56.3)	14 (35.9)	0.607
	Social drinker	64 (64)	41 (64.1)	23 (59)	
	Drinker	4 (4)	2 (3.3)	2 (5.1)	
Body mass index (kg/m^2)	<25	92 (92)	56	36 (92.3)	1.000
	>= 25 and <30	6 (6)	4	2 (5.1)	
	>=30	2 (2)	1	1 (2.6)	
AMH level(ng/ml)		3.4 (1.8-5.5)	4.0 (2.4-6.0)	3.2 (1.5-3.9)	0.038*
HbA1c (%)		5.2 (5.0-5.4)	5.2 (5.1-5.4)	5.1 (5.0-5.4)	0.725
Total cholesterol (mmol/L)		4.5 (3.9-5.1)	4.3 (3.8-5.1)	4.6 (4.2-5.0)	0.145
Triglycerides (mmol/L)		0.8 (0.6-1.1)	0.8 (0.6-1.1)	0.9 (0.6-1.1)	0.809
HDL – Cholesterol(mmol/L)		1.6 (1.4-1.9)	1.6 (1.3-1.8)	1.7 (1.5-1.9)	0.046*
LDL – Cholesterol(mmol/L)		2.3 (2.0-2.7)	2.2 (1.9-2.7)	2.4 (2.1-2.9)	0.299
Perceived Stress Scale score		17 (14-19)	17 (14-20)	17 (14-19)	0.766
MET (min per week)		1386 (742-3514)	1533 (792-3072)	1067 (692-3690)	0.496
MALE					
Age (years)		33.5 (30.3-35.9)	33.0 (29.3-35.4)	34.5 (31.8-37.2)	0.064
Education level	Secondary	15 (15)	7 (11.5)	8 (20.5)	0.257
	Tertiary or above	85 (85)	54 (88.5)	31 (79.5)	
Smoking	Never	76 (76)	44 (72.1)	32 (82.1)	0.532
	Quitted	14 (14)	10 (16.4)	4 (10.3)	
	Current	10 (10)	7 (11.5)	3 (7.7)	

Alcohol	Non-drinker	15 (15)	10 (16.4)	5 (12.8)	0.942
	Social drinker	74 (74)	44 (72.1)	30 (76.9)	
	Drinker	11 (11)	7 (11.5)	4 (10.3)	
Body mass index (kg/m^2)	<25	59 (59)	39 (63.9)	20 (51.3)	0.443
	>= 25 and <30	34 (34)	18 (29.5)	16 (41)	
	>=30	7 (7)	4 (6.6)	3 (7.7)	
HbA1c (%)		5.4 (5.2-5.5)	5.4 (5.2-5.5)	5.4 (5.1-5.7)	0.756
Total cholesterol (mmol/L)		4.6 (4.1-5.3)	4.7 (4.3-5.2)	4.5 (4.0-5.7)	0.454
Triglycerides (mmol/L)		1.4 (1.1-2.1)	1.6 (1.2-2.1)	1.3 (1.1-2.1)	0.613
HDL – Cholesterol(mmol/L)		1.3 (1.1-1.4)	1.2 (1.1-1.4)	1.3 (1.2-1.5)	0.039*
LDL – Cholesterol(mmol/L)		2.7 (2.1-3.2)	2.8 (2.3-3.2)	2.3 (1.9-3.3)	0.165
Sperm concentration (million per ml)		72.6 (36.6-121.8)	84.7 (36.2-144.5)	59.9 (41.8-96.0)	0.054
Sperm progressive motility (%)		45 (38-50)	46 (38-52)	43 (37-48)	0.162
Sperm morphology (%)		4 (3-5)	4 (3-6)	4 (2-5)	0.219
Total motile sperm count (million)		102.5 (49.6-200.4)	128.0 (62.8-245.3)	66.6 (36.4-186.3)	0.030*
Total motile normal morphology sperm count (million)		4.3 (1.6-8.3)	5.1 (1.9-12.0)	2.8 (1.4-5.5)	0.015*
Perceived Stress Scale score		16 (14-19)	16 (14-18)	16 (13-19)	0.977
MET (min per week)		1939 (1059-4119)	1739 (955-3687)	2388 (1386-6360)	0.247

Fisher's exact test and Mann-Whitney U test were used to compare categorical and continuous variables between groups respectively.
*Statistically significant

Out of the 100 analysed couples, 61 got pregnant within one year, with median time to pregnancy of 2 (1-5) months; 39 did not get pregnant in one year and fulfilled the definition of infertility. Those who conceived within one year, compared to those who did not, had significantly younger age and higher serum AMH level of the women, as well as total motile sperm count and TMNC.(Table 1)

Couples who conceived within one year also had lower HDL-cholesterol in both the female and male partner, although the magnitude of difference was very small. Other clinical parameters including coital frequency, age of men, body mass index, HbA1c, triglycerides, total cholesterol, LDL-cholesterol, PSS-10 score and physical activity (MET per week in the

IPAQ-SF) of the men and women were not statistically different between the two groups ($p>0.05$). The results are shown in Table 1.

Univariate logistic regression of factors predicting conception within one year by univariate analysis found that age, AMH and HDL-C level of the women, as well as TMNC, were significant factors predicting conception within one year (Table 2). When the age and serum AMH level of the women, and TMNC were entered for multivariate analysis, female's age and TMNC, but not serum AMH level of the women, were significant independent factors predicting conception within one year (Table 3).

TABLE 2: Univariate logistic regression analysis of factors predicting conception within one year

	Odds ratio (95% CI)	P value
Coital frequency at last follow up >=2 per week <2 per week	 Reference 0.71 (0.31-1.61)	0.408
Female		
Age (years)	0.83 (0.73-0.94)	0.004*
BMI (kg /M^2)	0.99 (0.86 -1.13)	0.825
AMH level (ng/ml)	1.20 (1.01-1.43)	0.038*
HbA1c (%)	0.72 (0.21-2.48)	0.599
Total cholesterol (mmol/L)	0.70 (0.40-1.21)	0.204
Triglycerides (mmol/L)	0.76 (0.31-1.88)	0.551
HDL – Cholesterol(mmol/L)	0.30 (0.10-0.95)	0.040*
LDL – Cholesterol(mmol/L)	0.81 (0.43-1.52)	0.503
Perceived Stress Scale score	1.01 (0.92-1.11)	0.817
MET (min per week)	1.00 (1.00-1.00)	0.515
Male		
Age (years)	0.92 (0.84-1.00)	0.055
BMI (kg /M^2)	0.95 (0.84-1.07)	0.413
HbA1c (%)	0.69 (0.23-2.07)	0.509
Total cholesterol (mmol/L)	1.05 (0.68-1.62)	0.835
Triglycerides (mmol/L)	1.18 (0.82-1.69)	0.369
HDL – Cholesterol(mmol/L)	0.23 (0.05-1.13)	0.070
LDL – Cholesterol(mmol/L)	1.28 (0.78-2.11)	0.323
Total motile sperm count (million)	1.00 (1.00-1.01)	0.152
Total motile normal morphology sperm count (million)	1.11 (1.02-1.20)	0.016*
Perceived Stress Scale score	1.00 (0.90-1.11)	0.954
MET (min per week)	1.00 (1.00-1.00)	0.496

*Statistically significant

Table 3: Predicting conception within one year by multivariate logistic regression analysis where the age and serum AMH level of the women and total motile normal morphology count of the men's semen were entered.

	Odds ratio (95% CI)	**P value**
Women's age (years)	0.87 (0.76-0.99)	0.032*
Serum AMH level (ng/ml)	1.13 (0.94-1.35)	0.189
Total motile normal morphology sperm count (million)	1.09 (1.00-1.19)	0.047*

*Statistically significant

Table 4 shows the correlation between time to pregnancy and clinical parameters in the women and men using Spearman's correlation. No parameter was significantly correlated with the time to pregnancy within one year. There was no significant difference in time to pregnancy (P= 0.643) between those having sexual intercourse twice per week or more and less than twice per week. Cox proportional-hazards regression analysis revealed that women's age (P=0.012), but not serum AMH nor TMNC, significantly contributed to prediction of time to pregnancy (Supplementary Table 1). The survival curve showing occurrence of pregnancy with time is shown in Supplementary Figure 1.

Table 4: **Spearman's correlation between time to pregnancy and the clinical parameters in the participants who conceived in one year (n =61)**

	Correlation coefficient	**P value**
Female		
Age (years)	0.23	0.075
BMI (kg/m^2)	0.15	0.249
AMH level (ng/ml)	-0.12	0.343
HbA1c (%)	-0.20	0.126
Total Cholesterol (mmol/L)	0.07	0.572
Triglycerides (mmol/L)	0.02	0.903
HDL Cholesterol (mmol/L)	0.08	0.560
LDL Cholesterol (mmol/L)	0.13	0.316
Perceived Stress Scale	-0.05	0.680
MET (Min per week)	0.24	0.091
Male		
Age (years)	0.16	0.217
BMI (kg/m^2)	0.13	0.327
HbA1c (%)	-0.25	0.060
Total Cholesterol (mmol/L)	0.08	0.541
Triglycerides (mmol/L)	0.15	0.240
HDL (mmol/L)	-0.06	0.638
LDL (mmol/L)	0.11	0.420
Total motile sperm count (million)	0.01	0.951
Total motile normal morphology sperm count (million)	-0.03	0.808
Perceived Stress Scale score	-0.01	0.920
MET (min per week)	-0.08	0.574

The areas under the ROC curve for women's age and TMNC in semen in predicting conception within one year were 0.66 (95% CI 0.55-0.77) and 0.65 (95% CI 0.54-0.75) respectively. That of the multivariate logistic regression model combing the two factors was 0.70 (95% CI 0.60-0.79).

Discussion

Findings from the present study suggested that the women's age and TMNC, but not serum AMH level, were significant factors predicting conception after trying for one year. A previous prospective observational study reported that 82% couples conceived during twelve cycles.[22] Another prospective study among those practising timed intercourse reported that 89.6% women conceived in one year.[23] However, an internet-based prospective study from Denmark reported a pregnancy rate of 69.6% only at 12 months.[24] In our study, however, only 61% of couples conceived in one year. The apparently lower figure could be due to the relatively low coital frequency in our population, even among pregnancy planners. Less than 50% of our cohort had sexual intercourse at least two times per week. In a study on Hong Kong women attending family planning and pre-pregnancy check-up services in 2007-2009, 18.1% had intercourse less than 12 times in a year.[25]

Increasing women's age was demonstrated to be a good predictor of reduced fecundability, while men's age was not associated with fecundability after adjustment for the women's age.[26] In the 501 couples of the Longitudinal Investigation of Fertility and the Environment (LIFE) study, men's age was associated with fecundability.[27] However, the women's age was not controlled for in that study, and it could be that older men merely had older female partners. Our study suggested that older women, but not the men, were associated with reduced fecundability. Reproductive aging in men generally occurs at the more extreme end of age.[28] Given that majority of our study participants were in the young range (all men were aged <=50 years), it is not unexpected that men's age was not observed to have independent influence on fecundability.

Semen parameters have been reported to be associated with time to pregnancy.[27, 29] In line with that, reduced TMNC was found to be significantly associated with reduced fecundability in our study. Obviously, this determines the number of sperms available at the site of fertilisation after the sexual act. However, this is not the sole determinant as other factors such as coital frequency would be important as well.

It is worth to note that fecundability is subjected to multifactorial influence. The predictive value of either the women's age or TMNC alone or in combination was just modest, as shown by the c-statistics in the ROC curves, despite statistical significance of the individual factors.

Steiner *et al*. reported that AMH was associated with day-specific probability of pregnancy.[5] A study of 750 women from the same group found that low AMH was not associated with reduced fecundability.[9] Other studies involving younger healthy pregnancy planners found that AMH did not predict natural fertility.[6-7,10] An observational study of 87 women who had a live birth from planned natural pregnancy found that AMH was not related to the time to pregnancy.[8] The women in our study were younger, where only 17 percent were aged 35 years or above, and our result concurred with those studies involving younger women.

Cholesterol is a substrate for steroidogenesis and hence may impact on fertility .[30] The LIFE study found that higher free cholesterol was associated with reduced fecundability independent of BMI.[15] A secondary analysis of the Effects of Aspirin in Gestation and Reproduction trial in 2017 also found that reduced fecundability was associated with all lipoprotein abnormalities including low HDL-cholesterol.[31] Our results revealed that lower HDL-cholesterol was significantly associated with conception at one year. However, the magnitude of difference was extremely small, and is unlikely to be clinically meaningful. We also studied the effect of HbA1c, a glycaemic index, on fecundability, which has not been reported in the literature. No significant effect of HbA1c on fecundability was suggested in our cohort. This could be limited by the fact that none in our cohort had abnormal HbA1c at or above 6.0%.

A previous study reported that a total MET of 1200-1740 minutes per week was associated with highest fecunability.[16] Although couples who conceived in 1 year in our cohort had similar MET-min per week, we could not demonstrate a significant effect of physical activity on fecundability, probably because majority in our cohort were not doing vigorous physical activity.

The main strength of our present study is that it was a prospective study with one year follow up and both the male and female partners of the couples were closely followed up so as to avoid recall bias. The subjects were recruited among pregnancy planners in the community, in contrast to some previous studies which targeted on couples attending infertility clinics. However, our sample size was only modest, and it was just adequate to confer adequate power for our main analyses. A larger further study with adequate power to establish and validate a prediction model by the women's age and total motile normal morphology sperm count would be worthwhile. Another limitation was that semen parameters and lifestyle factors such as smoking, exercise and stress level may fluctuate or change with time. We only assessed these parameters at recruitment which may not represent the whole course of the follow-up period. The fact that majority of the couples were non-smoker and with normal BMI may make the comparison of these parameters difficult and limiting its predicting power, and our findings cannot be extrapolated to couples with more extreme BMIs.

In conclusion, women's age and total motile normal morphology sperm count are significant independent predictors of conception within one year. No parameter can predict the time to pregnancy within one year. This finding can aid preconceptional counselling of couples who are ceasing contraception in preparation for pregnancy.

Contributors

HWRL, WSBY, PCH and EHYN conceived and designed the study. MTL, HWRL, CYGW and EHYN supervised subject recruitment and follow-up. MTL analysed the data, interpreted the results and drafted the manuscript. All authors provided critical revisions and approved the final manuscript prior to submission.

Acknowledgements

The authors would like to thank Ms. Celia Ng and Ms. Wylie Wong for co-ordinating subject recruitment and follow-up. We would also express our gratitude to all staff members of the Family Planning Association of Hong Kong who facilitated the conduct of this study, and to Ms. Benency Po-Chau Wong and Dr. Angel Chan for performing the biochemical assays. Last but not least, we thank all the couples who participated in this study.

Funding/support:

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors. It was supported by internal research funding of the Department of Obstetrics and Gynaecology, The University of Hong Kong.

Ethical approval

Ethics approval was obtained from both the Institutional Review Board of the University of Hong Kong / Hospital Authority Hong Kong West Cluster. Approval was also obtained from the Health Services Subcommittee, Family Planning Association of Hong Kong.

Conflicts of Interest:

All authors have no conflicts of interest to disclose.

References:

1. Zegers-Hochschild F, Adamson GD, Dyer S, *et al*. The international glossary on infertility and fertility care, 2017. *Hum Reprod* 2017;32(9):1786-1801.
2. Sobotka T. Is lowest-low fertility in Europe explained by the postponement of childbearing? *Population and Development Review* 2004;30:195-220.
3. La Marca A, Stabile G, Artenisio AC, *et al*. Serum anti-mullerian hormone throughout the human menstrual cycle. *Hum Reprod* 2006; 21(12): 3103-3107.
4. Iliodromiti S, Kelsey TW, Wu O, *et al*. The predictive accuracy of anti-Müllerian hormone for live birth after assisted conception: a systematic review and meta-analysis of the literature. *Hum Reprod Update* 2014; 20(4):560-570.
5. Steiner AZ, Herring AH, Kesner JS, *et al*. Antimullerian hormone as a predictor of natural fecundability in women aged 30-42 years. *Obstet Gynecol* 2011; 117(4): 798-804.
6. Hagen CP, Vestergaard S, Juul A, *et al*. Low concentration of circulating antimullerian hormone is not predictive of reduced fecundability in young healthy women: a prospective cohort study. *Fertil Steril* 2012; 98(6):1602-1608.
7. Depmann M, Broer SL, Eijkemans MJC, *et al*. Anti- Mullerian hormone does not predict time to pregnancy: results of a prospective cohort study. *Gynecol Endocrinol* 2017;33(8):644-8.
8. Streuli I, de Mouzon J, Paccolat C, *et al*. AMH concentration is not related to effective time to pregnancy in women who conceive naturally. *Reprod Biomed Online* 2014; 28(2):216-24.

9. Steiner AZ, Pritchard D, Stanczyk FZ, *et al*. Association between biomarkers of ovarian reserve and infertility among older women of reproductive age. *JAMA* 2017;318(14):1367-76.

10. Korsholm AS, Petersen KB, Bentzen JG, *et al*. Investigation of anti-mullerian hormone concentrations in relation to natural conception rate and time to pregnancy. *Reprod Biomed Online* 2018;36(5):568-75.

11. World Health Organisation. WHO Laboratory Manual for the Examination and Processing of Human Semen, 5th ed. Geneva: WHO Press; 2010.

12. Esteves SC, Zini A, Aziz N, *et al*. Critical appraisal of World Health Organization's new reference values for human semen characteristics and effect on diagnosis and treatment of subfertile men. *Urology* 2012; 79(1): 16-22.

13. Gesink Law DC, Maclehose RF, Longnecker MP, *et al*. Obesity and time to pregnancy. *Hum Reprod* 2007;22(2):414-20.

14. Ramlau-Hansen CH, Thulstrup AM, Nohr EA, *et al*. Subfecundity in overweight and obese couples. *Hum Reprod* 2007;22(6):1634-7.

15. Schisterman EF, Mumford SL, Browne RW, *et al*. Lipid concentrations and couple fecundity: the LIFE study. *J Clin Endocrinol Metab* 2014; 99(8): 2786-94.

16. Wise LA, Rothman KJ, Mikkelsen EM, *et al*. A prospective cohort study of physical activity and time to pregnancy. *Fertil Steril* 2012; 97(5):1136-42.

17. Wesselink AK, Hatch EE, Rothman KJ, *et al*. Perceived stress and fecundability: a preconception cohort study of North American couples. *Am J Epidemiol* 2018;187(12):2662-71.

18. Craig CL, Marshall AL, Sjostrom M, *et al*. International physical activity questionnaire: 12-country reliability and validity. *Med Sci Sports Exerc* 2003; 35(8):1381-95.

19. Macfarlane DJ, Lee CC, Ho EY, *et al*. Reliability and validity of the Chinese version of IPAQ (short, last 7 days). *J Sci Med Sport* 2007; 10(1):45-51.

20. Cohen S, Williamson G. Perceived stress in a probability sample of the United States. In: Spacapam S, Oskamp S (eds). The Social Psychology of Health: Claremont Symposium on Applied Social Psychology. Newbury Park (CA): SAGE; 1988. p 31–66.

21. Li HWR, Wong BPC, Ip WK, Yeung WSB, Ho PC, Ng EHY. Comparative evaluation of three new commercial immunoassays for anti-Müllerian hormone measurement. Hum Reprod 2016;31(12):2796-2802.

22. Zinaman MJ, Glegg ED, Brown CC, *et al*. Estimates of human fertility and pregnancy loss. *Fertil Steril* 1996;65(3):503-9.

23. Gnoth C, Godehardt D, Godehardt E, *et al*. Time to pregnancy: results of the German prospective study and impact on the management of infertility. *Hum Reprod* 2003; 18(9): 1959-66.

24. Wise LA, Rothman KJ, Mikkelsen EM, *et al*. An internet-based prospective study of body size and time-to-pregnancy. *Hum Reprod* 2010; 25(1):253-64.

25. Lo SST, Kok WM. Sexual behavior and symptoms among reproductive age Chinese women in Hong Kong. *J Sex Med* 2014;11(7):1749-56.

26. Wesselink AK, Rothman KJ, Hatch EE, *et al*. Age and fecundability in a North American preconception cohort study. *Am J Gynecol* 2017; 217(6):667.e1-667.e8.

27. Buck Louis GM, Sundaram R, Schisterman EF, *et al*. Semen quality and time to pregnancy: the longitudinal investigation of fertility and the environment study. *Fertil Steril* 2014;101(2):453-62.

28. Lai SF, Li RH, Yeung WS, *et al*. Effect of paternal age on semen parameters and live birth rate of in-vitro fertilisation treatment: a retrospective analysis. *Hong Kong Med J* 2018;24(5):444-450.

29. Bonde JP, Ernst E, Jensen TK, *et al*. Relation between semen quality and fertility: a population-based study of 430 first-pregnancy planners. *Lancet.* 1998;352(9135):1172-7.

30. DeAngelis AM, Roy-O'Reilly M, Rodriguez A, *et al*. Genetic alterations affecting cholesterol metabolism and human fertility. *Biol Reprod* 2014;91(5):117,1-10

31. Pugh SJ, Schisterman EF, Browne RW, *et al*. Preconception maternal lipoprotein levels in relation to fecundability. *Hum Reprod* 2017;32(5):1055-63.

Supplementary Table 1. Cox proportional-hazards regression analysis on women's age, serum AMH and TMNC as predictors of time to pregnancy.

Covariate	b	Exp(b) (95% confidence interval)	P
Women's age (years)	-0.11	0.90 (0.83 – 0.98)	0.012
Serum AMH (ng/ml)	0.07	1.07 (0.97 – 1.17)	0.160
TMNC (million)	0.03	1.03 (1.00 – 1.07)	0.067

Supplementary Figure 1. Survival curve showing conception probability with time.