Effect of paternal age on semen parameters and live birth rate of in-vitro fertilisation treatment: a retrospective analysis

SF Lai *, Raymond HW Li, William SB Yeung, Ernest HY Ng

ABSTRACT

Objective: To determine the effect of paternal age on semen parameters and the live birth rate from in-vitro fertilisation (IVF) treatment.

Methods: We performed a retrospective cohort study of couples undergoing a first IVF cycle between 2004 and 2014 in a tertiary assisted reproduction centre in Hong Kong.

Results: We analysed 3549 cases. Paternal age ≥40 years was negatively correlated with semen volume, progressive motility, total motility and total normal motile count (P<0.005) and positively correlated with sperm concentration (P<0.001). There was no correlation with sperm count, normal morphology, or total motile count. Subgroup analyses in Chinese men only and in men with normal versus abnormal semen parameters showed the same correlations. Paternal age was positively associated with maternal age (P<0.001) and miscarriage (P=0.006), and negatively associated with ongoing pregnancy and live birth (P<0.001). Logistic regression showed that maternal age, total number of oocytes retrieved, and number of embryos transferred were significant factors which independently predicted the likelihood of live birth from IVF (all P<0.001).

Conclusion: Paternal age was negatively correlated with some semen parameters, which showed a significant decline after age 40 years. However, paternal age is not predictive of the live birth from IVF treatment.

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New knowledge added by this study
- Paternal age negatively correlates with some semen parameters.
- Paternal age is not an independent predictor of the likelihood of a live birth from in-vitro fertilisation treatment, after controlling for the maternal age, the number of oocytes retrieved, and the number of embryos transferred.

Implications for clinical practice or policy
- Infertile couples can be counselled that although there is a decline in some semen parameters with paternal age ≥40, the live birth rate of in-vitro fertilisation treatment depends primarily on maternal age, number of oocytes retrieved and number of embryos transferred, but not on paternal age.

Introduction

In recent years, marriages and pregnancies are occurring later and later in life, which was confirmed in a recent large-scale analysis conducted in the US.[1] Local statistics in Hong Kong support this trend, as the median age at first marriage for both sexes has risen over the past 20 years.[2,3] Extensive data are available on the adverse effects of increasing maternal age on in-vitro fertilisation (IVF) outcomes[4-6] but little information was on the adverse effects of increasing paternal age.

Two systematic reviews have looked at the effect of paternal age on semen parameters and assisted reproduction outcomes.[7,8] Dain et al[7] demonstrated that paternal age did not affect pregnancy, miscarriage, and live birth rates. The authors also revealed that semen volume decreased with paternal age, but sperm motility, concentration and morphology did not. Later, in another systematic review[8] of 12 studies on oocyte donor cycles, the same group showed that advancing paternal age is not associated with adverse outcomes, including pregnancy and live birth rates. They also showed that, except for volume and possibly motility, sperm characteristics such as concentration and morphology did not alter with age. However, most papers studied did not report the live birth rate, which is the most important clinical outcome for the

patients involved.

The existing information regarding the adverse effects of increasing paternal age on semen parameters and IVF outcomes is mostly from Western populations.[7-9] Therefore, we conducted this study to determine the effect of paternal age on semen parameters and on the live birth rate of IVF treatment in the Hong Kong population, which is mainly of Chinese ethnicity.

Methods

Subject inclusion and exclusion

We retrieved all first IVF cycles carried out between 2004 and 2014 at the Centre of Assisted Reproduction and Embryology, The University of Hong Kong–Queen Mary Hospital from the Assisted Reproduction Clinical Database of the Centre. Only the first IVF cycles using ejaculated semen were included for analysis, to avoid any potential bias. Cases requiring preimplantation genetic diagnosis or using donor sperm or surgically retrieved sperm were excluded from the study.

Ovarian stimulation

Details of the treatment protocol has been previously described.[10] In brief, women received ovarian stimulation following either the long gonadotropin-releasing hormone (GnRH) agonist or antagonist protocol. A baseline ultrasound was performed on day 2 to 3 of the cycle to exclude pre-existing ovarian cysts. Serum oestradiol (E2) concentration was measured to confirm the basal level. In the long GnRH agonist protocol, intranasal buserelin acetate (Suprecur; Sanofi, France) was started on day 21 of the preceding cycle at 150 μg 4 times a day and continued until the day of ovulation trigger. In the GnRH antagonist protocol, subcutaneous injection of ganirelix 0.25 mg (Orgalutran; Organon, The Netherlands) or cetrorelix 0.25 mg (Cetrotide; Merck Serono, Germany) was started on day 6 after gonadotropin injection until the day of ovulation trigger. Human menopausal gonadotropin (hMG, Menogon; Ferring, Switzerland) or recombinant follicle-stimulating hormone (Gonal-f [Merck Serono, Germany] or Puregon [Organon, The Netherlands]) injection was started at a dosage as determined by the antral follicle count. Ovarian response was monitored by transvaginal ultrasound. Human chorionic gonadotropin (hCG; Profasi [10 000 units; MSD, US] or Ovidrel [250 μg; Merck Serono, Switzerland]) was given to trigger the final oocyte maturation when there were at least three follicles ≥16 mm in diameter, of which one follicle was ≥18 mm. Triptorelin 0.2 mg (Decapeptyl; Ferring, Switzerland) was used to replace hCG if serum E2 concentration was >25 000 pmol/L, if more than 15 follicles were ≥14 mm in diameter on the day of hCG

administration, or when the patient had evidence of ovarian hyperstimulation syndrome. Transvaginal ultrasound-guided oocyte retrieval was performed 34 to 36 hours after the ovulatory dose of hCG or triptorelin injection. Instruction on abstinence of sex for 2 to 7 days was given prior to submission of any semen sample. Fresh ejaculated semen samples were evaluated according to World Health Organization guidelines[11,12] and the same protocol (including the strict criteria for assessing sperm morphology) was adopted throughout the period covered in this study.

Intracytoplasmic sperm injection (ICSI) was performed when the normal morphology of a recent semen sample was <3% or the total motile sperm count after sperm preparation was <0.2 million. The same criteria for ICSI versus conventional insemination was adopted throughout the study period. Fertilisation was assessed 16 to 20 hours after insemination. Embryo transfer was performed under ultrasound guidance 2 days after retrieval. Up to three embryos were transferred before 2006 and a maximum of two embryos were transferred after 2006. Luteal phase was supported with vaginal progesterone pessaries (Cyclogest 400 mg twice a day [Cox Pharmaceuticals, Barnstaple, United Kingdom] or Endometrin 100 mg twice a day [Ferring, Switzerland]) or intramuscular injection of hCG 1500 units every 6 days for two doses. Urine pregnancy test was performed 16 days after embryo transfer. Where the pregnancy test was positive, ultrasound scans were performed at 6 and 8 weeks of gestation to confirm fetal viability and number.

Total motile count (TMC) was defined as total sperm count with progressive motility (total count

男性年齡對精液檢驗參數和體外受精活產率的影響：回顧研究

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引言：檢視男性年齡對精液檢驗參數和體外受精活產率的影響。
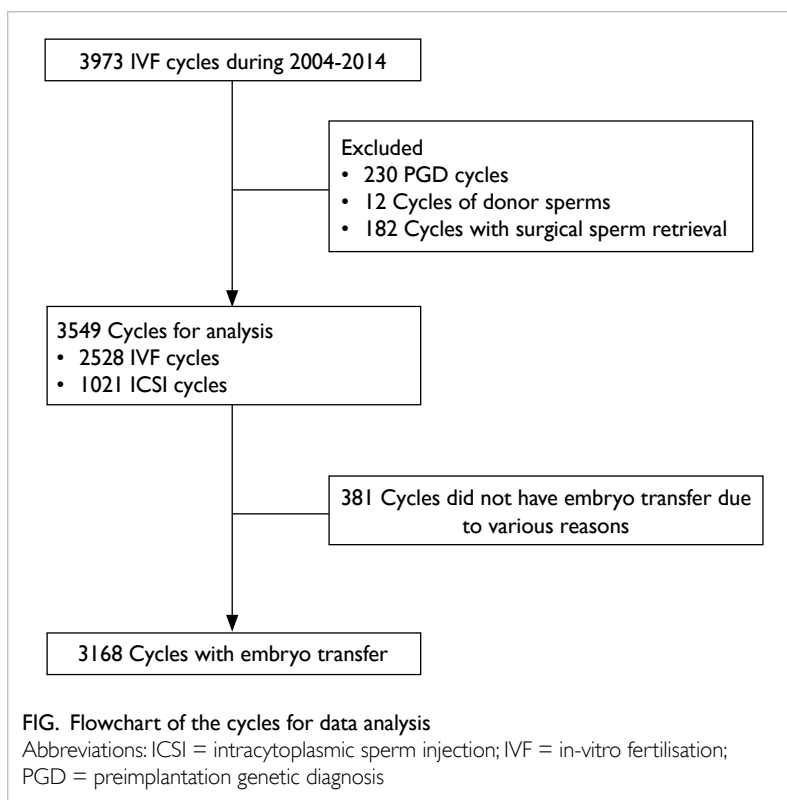
方法：回顧隊列研究，對象為2004年至2014年間於香港一所生殖醫學中心接受首次體外受精的夫婦。

結果：根據3549對夫婦的數據分析，40歲或以上男性其精子濃度較高（P<0.001），但其精液量、前向活動精子百分比、總活動精子百分比和總正常前向活動精子量均較低（P<0.005）；但男性年齡層與精子數量、正常精子形態或總前向活動精子量無關。於單純華裔男性或具有正常或異常精液檢驗參數的分組分析所得出結論也與上述無異。男性年齡與妻子年齡（P<0.001）和流產率（P=0.006）呈正關聯，但與體外受精持續懷孕率及活產率則呈反關聯（P<0.001）。邏輯迴歸分析顯示妻子年齡、獲卵總數和所移植胚胎數目是預測體外受精的活產機會之獨立因子（P<0.001）。

結論：男性年齡與部份精液檢驗參數呈反關聯，尤其40歲後更為明顯，惟其不能預測體外受精之活產機會。

FIG. Flowchart of the cycles for data analysis
Abbreviations: ICSI = intracytoplasmic sperm injection; IVF = in-vitro fertilisation; PGD = preimplantation genetic diagnosis

× % with progressive motility). Total normal motile count (TNMC) was defined as sperm count with progressive motility and normal morphology (total count × % with progressive motility × % with normal morphology).

Pregnancy was defined by a positive urine or serum hCG test. Miscarriage was defined as a pregnancy which became non-viable before 24 weeks of gestation; this included biochemical pregnancies and miscarriages before 24 weeks. An ongoing pregnancy was defined as presence of intrauterine sac(s) with positive fetal heart pulsation at 8 weeks of gestation. A live birth was defined as the complete expulsion or extraction from a woman of a conceptus after 24 completed weeks of gestational age which, after such separation, showed evidence of life.

Statistical analyses

The key outcomes of this study were live birth and semen parameters including volume, concentration, count, progressive motility, total motility, normal morphology, TMC, and TNMC.

Statistical analysis was performed using SPSS Windows version 24.0 (IBM Corp, Armonk [NY], US) and MedCalc (Version 12, Belgium). Paternal age of our cohort was not normally distributed as shown by Kolmogorov-Smirnov test (P<0.001). Therefore, non-parametric tests were used for analysis. The Mann-Whitney U or Kruskal-Wallis H tests were used to compare continuous variables

among groups. Spearman's correlation was used to determine correlations between continuous variables. Chi squared test was used for analysis of categorical variables. Logistic regression analysis was used to examine factors predicting live birth, first by univariate analysis of individual variables, and those factors showing significance were subsequently entered into multivariate analysis. A two-tailed value of P<0.05 was considered statistically significant.

Results

A total of 3973 first IVF cycles were performed during the study period (Fig). Of these, 424 cases were not selected (230 with a preimplantation genetic diagnosis, 12 using donor sperm, and 182 using surgically retrieved sperm). In ICSI cases, if the sperm concentration was <3 million/mL, morphology would not be evaluated (n=207). Sperm concentration, count and/or normal morphology could not be evaluated in the fresh semen samples of 84 men with severe male factors. Hence, TNMC was only available in 3258 cases.

Cohort demographics are shown in Table 1. The causes of infertility were as follows: tuboperitoneal factor (n=620; 17.5%), endometriosis (n=294; 8.3%), male factor (n=1483; 41.8%), unexplained (n=603; 17.0%), and mixed factors (n=549; 15.5%). Among those analysed, conventional insemination was performed in 2528 (71.2%) cycles and ICSI was required in 1021 (28.8%) cycles. There were 381 (10.7%) cases which did not have fresh embryo transfer for various reasons, leaving 3168 cases with fresh embryo transfer. There were 1613 pregnancies, giving a pregnancy rate of 50.9% per transfer. Of these pregnancies, 241 (14.9%) miscarried before 8 weeks of gestation. An additional 43 (2.7%) cases miscarried after 8 weeks of gestation. Four (0.2%) women terminated their pregnancy due to congenital abnormalities (n=3) or for social reasons (n=1). Five (0.3%) pregnancies ended in intrauterine death.

Paternal age was negatively correlated with the semen volume, progressive motility, total motility, and TNMC (P<0.005), as shown in Table 2. Paternal age was positively correlated with sperm concentration (P<0.001). Paternal age was not correlated with sperm count, normal morphology, or TMC.

We divided paternal age into two groups (<40 years and ≥40 years) and analysed the difference in semen parameters using the Mann-Whitney U test. Results showed a significant decline in semen volume, progressive motility, total motility and TNMC (P<0.005), but a significant increase in sperm concentration for age ≥40 years (P<0.001). There was no significant difference in sperm count, normal morphology, TMC or fertilisation rate between the age-groups (P>0.05). These findings support the correlations shown in Table 2.

The analyses were repeated in the subset including Chinese men only (n=3394, 95.6%), and showed similar correlations between age and semen parameters and IVF outcomes.

When men were grouped into those with normal and abnormal semen parameters, the negative correlation of paternal age with semen volume, progressive motility, total motility and TNMC and the positive correlation with sperm concentration remained the same as that for the whole cohort.

Paternal age was not significantly associated with the fertilisation rate in the conventional IVF group (P=0.786), in the ICSI group (P=0.801), or overall (P=0.810) as shown in Table 2. Paternal age was positively correlated with maternal age (Spearman's correlation coefficient: 0.487; P<0.001). Paternal age was significantly lower in those who attained pregnancy, ongoing pregnancy and live birth compared with those who did not (P<0.001; Mann-Whitney *U* test). In contrast, paternal age was significantly higher in cases that ended in miscarriage than in those that achieved live birth (P=0.006; Mann-Whitney *U* test).

The clinical and demographic characteristics of patients with or without a live birth in the first IVF cycles are compared in Table 3. Women who had a live birth were significantly younger (median 35.0 vs 36.0 years), had a younger partner (median 37.0 vs 38.0 years), higher antral follicle count (median 11 vs 8), more oocytes retrieved (median 9 vs 7), and had double embryo transfer (86.5% vs 74.6%). There was no statistically significant difference in the maternal BMI nor in TNMC between these two groups. The analysis showed similar findings between pregnant versus non-pregnant groups and between those with ongoing pregnancy and those without.

Logistic regression on individual variables by univariate analysis was used to analyse the prediction on the live birth in the first IVF cycle. Only paternal age, maternal age, total number of oocytes retrieved, and number of embryos transferred were found to be significant predictors. On combining these variables in a multivariate analysis, maternal age, total number of oocytes retrieved and number of embryos transferred, but not paternal age, were the significant factors which independently predicted the likelihood of live birth in the first IVF cycles after controlling for the others (P<0.001), as shown in Table 4.

Discussion

This is the first large-scale study on the effect of paternal age on semen parameters and IVF outcomes in our region, with the majority of patients being Chinese. Nearly 4000 first IVF cycles were analysed. Most previous studies have reported on oocyte donation models.[7,8] Our cohort is the largest sample

TABLE 1. Demographics of the cohort

	Data*
Paternal age (years)	38 (35-42)
Semen volume (mL)	3.1 (2.3-4.2)
Sperm concentration (million/mL) (n=3470)	47.9 (20.0-87.0)
Sperm count (million) (n=3470)	139.9 (56.3-270.3)
Progressive motility (%)	42 (28-53)
Total motility (%)	50 (37-60)
Normal morphology (%) (n=3260)	5 (3-8)
TMC (million) (n=3469)	58.6 (17.5 - 125.2)
TNMC (million) (n=3260)	3.1 (0.8-8.2)
Maternal age (years)	36 (33-38)
Maternal BMI (kg/m²) (n=3139)	21.4 (19.8-23.3)
Type of infertility (n=3427)	
Primary	2309 (67.4)
Secondary	1118 (32.6)
Antral follicle count (n=3325)	9 (6-14)
Total No. of oocytes	8 (5-12)
No. of embryos transferred (n=3549)	
0	381 (10.7)
1	638 (18.0)
2	2512 (70.8)
3	18 (0.5)
Pregnancy per transfer (n=3168)	1613 (50.9)
Miscarriage rate (n=1613)	284 (17.6)
Ongoing pregnancy per transfer (n=3168)	1311 (41.4)†
Live birth per transfer (n=3168)	1227 (38.7)†

Abbreviations: BMI = body mass index; TMC = total motile count; TNMC = total normal motile count
* Data are shown as median (interquartile range) or No. (%) of patients
† Including 2 cases of heterotopic pregnancy

TABLE 2. Correlation of paternal age with semen parameters and IVF parameters

	Spearman's correlation coefficient	P value
Semen volume	-0.116	<0.001
Sperm concentration	0.064	<0.001
Sperm count	0.011	0.506
Progressive motility	-0.103	<0.001
Total motility	-0.122	<0.001
Normal morphology	-0.031	0.077
TMC	-0.031	0.07
TNMC	-0.053	0.002
Maternal age	0.487	<0.001
Fertilisation rate		
Overall	-0.004	0.810
Conventional insemination group	-0.005	0.786
ICSI group	0.008	0.801

Abbreviations: ICSI = intracytoplasmic sperm injection; IVF = in-vitro fertilisation; TMC = total motile count; TNMC = total normal motile count

TABLE 3. Comparison of demographic and clinical characteristics of patients with or without a live birth in the first IVF cycle*

	No live birth in the first IVF cycle (n=1909)	Live birth in the first IVF cycle (n=1227)†	P value‡
Paternal age (years)	38.0 (35.0-42.0)	37.0 (35.0-41.0)	<0.001
Semen volume (mL)	3.1 (2.2-4.2)	3.2 (2.3-4.2)	0.696
Sperm concentration (million/mL)	49.0 (21.1-88.3)	47.8 (18.7-87.0)	0.127
Sperm count (million)	144.0 (62.2-276.9)	138.7 (52.7-269.6)	0.188
Progressive motility (%)	42 (28-53)	42 (28-53)	0.804
Total motility (%)	50 (37-60)	50 (37-60)	0.920
Normal morphology (%)	5 (3-8)	5 (3-8)	0.376
TMC (million)	60.1 (18.2-126.2)	56.0 (15.2-126.6)	0.244
TNMC (million)	3.2 (0.8-8.6)	3.2 (0.7-7.7)	0.432
Maternal age (years)	36.0 (34.0-38.0)	35.0 (33.0-37.0)	<0.001
Maternal BMI (kg/m²)	21.3 (19.8-23.3)	21.4 (19.8-23.5)	0.379
Antral follicle count	8.0 (5-13)	11.0 (6-16)	<0.001
Total No. of oocytes	7.0 (4-11)	9.0 (6-12)	<0.001
No. (%) of embryos per transfer			<0.001§
1	469 (24.6)	163 (13.3)	
2	1425 (74.6)	1061 (86.5)	
3	15 (0.8)	3 (0.2)	

Abbreviations: BMI = body mass index; IVF = in-vitro fertilisation; TMC = total motile count; TNMC = total normal motile count
* Data are shown as median (interquartile range), unless otherwise specified. Only cases with embryo transfer were included
† Missing data in 32 cases. There were 2 cases of heterotopic pregnancy
‡ Mann-Whitney U test
§ Chi squared test

TABLE 4. Logistic regression analysis of factors for prediction of a live birth in the first IVF cycle*

Factor	B	Exp(B), 95% CI	P value
Paternal age	-0.005	0.995 (0.979-1.010)	0.497
Maternal age	-0.124	0.883 (0.860-0.907)	<0.001
Total No. of oocytes	0.046	1.047 (1.031-1.063)	<0.001
No. of embryos transferred	0.717	2.048 (1.678-2.499)	<0.001

Abbreviations: CI = confidence intervals; IVF = in-vitro fertilisation
* Only cases with embryo transfer were included; 32 cases with missing delivery details were not included in the analysis

size in the literature based on autologous oocytes and fresh semen samples, with live birth as one of the key outcomes. Logistic regression analysis was employed to differentiate the factors affecting live birth in the first IVF cycles.

Our results show that paternal age was negatively correlated with semen volume, progressive motility, total motility, TNMC but not sperm count, normal morphology nor TMC. The positive correlation between paternal age and sperm concentration might be explained by the decrease in semen volume with age, resulting in an apparent raised sperm concentration. An increase in sperm concentration was also found in a recent study conducted on a similar scale.[13] Other studies have mostly reported either no significant change[14,15] or a decrease in sperm concentration.[16] Although increasing paternal age was not associated with any significant change in normal morphology, the associated significant reduction in progressive motility contributed to an overall negative impact on TNMC. These composite parameters represent the population of sperm relevant to natural fertility. The decline in overall sperm quality with paternal age is likely due to the decline in testicular function.[17] The number of Leydig cells,[18] Sertoli cells[19] and germ cells decreases with paternal age.[20] Despite the overall decrease in various semen parameters with age, we found that the fertilisation rate was not significantly reduced with increasing age; this is compatible with most previous studies.[7,8]

Various age cut-offs have been suggested in the literature for defining advanced paternal age but the most frequently used cut-off is age 40 years at the time of conception.[21] Both the American Society for Reproductive Medicine and the British Andrology Society recommend that the sperm donor should be age <40 years.[22,23] Our results show that there is an inverse relationship in semen parameters with paternal age using the cut-off of age 40 years.

Multiple studies have shown that advanced paternal age is associated with a significant increase in DNA fragmentation[24] which in turn is associated with higher rate of IVF failure.[25] As men age, the sperm chromatin integrity weakens and sperm DNA fragmentation increases.[26] Fecundability has been shown to decrease with increased abnormal sperm chromatin percentage.[27] The molecular ageing process has been shown to induce changes in reproductive hormone profiles, decreasing in sperm quality parameters, and contributing to male infertility.[28]

On univariate analysis, paternal age was associated inversely with live birth. However, paternal age was also positively correlated with maternal age, meaning that younger men usually have younger partners and vice versa. Logistic regression analysis indicated that maternal age but not paternal age was an independent predictor of the likelihood of live birth, in contrast to some previous studies.[29,30] Paternal age is likely a surrogate marker of maternal age and does not have a direct effect on IVF outcomes.

The analyses were repeated in the subset including Chinese men only, revealing similar findings on semen parameters and IVF outcomes. However, we are cautious of the interpretation of this finding as the majority of our cohort was Chinese.

Although different ovarian stimulation protocols and different medications were used throughout the study period, meta-analyses have shown that there are no differences in the live birth rates between the long GnRH agonist protocol and the GnRH antagonist protocol, between the use of hMG and recombinant follicle-stimulating hormone, nor among the different types of progestogens used for luteal phase support in terms of pregnancy outcomes.[31-34]

Our study has some limitations. We followed the delivery details of most patients; only 32 patients were lost to follow-up. However, we do not have the information on any congenital abnormalities of the live-born or long-term data of the babies born via IVF/ICSI. The sample size of the study is also too small to evaluate these outcomes. Hence, we cannot evaluate the long-term effects of advanced paternal age on their children, if any. However, multiple studies have shown that advanced paternal age is associated with increased incidence of schizophrenia,[35,36] autism,[37,38] and genetic conditions such as achondroplasia and Apert's syndrome, despite young maternal age in their children.[39,40] Although the semen parameters analysed were based on the one-off semen sample provided for insemination, semen parameters are known to vary with time. We do not have data on paternal body weight, smoking and drinking habits, medical condition, hormone levels or exact time of abstinence. The range of paternal age was also relatively narrow in this cohort.

The present study examined patients who had undergone IVF treatment. This study represents only one subset of infertile patients. Patients undergoing other forms of fertility treatment such as intrauterine insemination would be a valuable subject for further studies.

Conclusion

Paternal age is negatively correlated with some semen parameters, which show a significant decline after 40 years. Maternal age, the number of oocytes retrieved, and the number of embryos transferred, but not paternal age are predictive of live birth from IVF treatment.

References

1. Khandwala YS, Zhang CA, Lu Y, Eisenberg ML. The age of fathers in the USA is rising: an analysis of 168 867 480 births from 1972 to 2015. Hum Reprod 2017;32:2110-16.
2. Census and Statistics Department, Hong Kong SAR Government. Hong Kong monthly digest of statistics (January 2015). 15 Jan 2015. Available from: https://www.censtatd.gov.hk/fd.jsp?file=B10100022015MM01B0100.pdf&product_id=B1010002&lang=1. Accessed 25 Oct 2017.
3. Census and Statistics Department, Hong Kong SAR Government. Hong Kong monthly digest of statistics (December 2015). 15 Dec 2015. Available from: https://www.censtatd.gov.hk/fd.jsp?file=B10100022015MM12B0100.pdf&product_id=B1010002&lang=1. Accessed 25 Oct 2017.
4. McLernon DJ, Steyerberg EW, Te Velde ER, Lee AJ, Bhattacharya S. Predicting the chances of a live birth

after one or more complete cycles of in vitro fertilisation: population based study of linked cycle data from 113 873 women. BMJ 2016;355:i5735.

5. Toner JP, Coddington CC, Doody K, et al. Society for Assisted Reproductive Technology and assisted reproductive technology in the United States: a 2016 update. Fertil Steril 2016;106:541-6.

6. Bhattacharya S, Maheshwari A, Mollison J. Factors associated with failed treatment: an analysis of 121 744 women embarking on their first IVF cycles. PLoS One 2013;8:e82249.

7. Dain L, Auslander R, Dirnfeld M. The effect of paternal age on assisted reproduction outcome. Fertil Steril 2011;95:1-8.

8. Sagi-Dain L, Sagi S, Dirnfeld M. Effect of paternal age on reproductive outcomes in oocyte donation model: a systematic review. Fertil Steril 2015;104:857-65.e1.

9. Sagi-Dain L, Sagi S, Dirnfeld M. The effect of paternal age on oocyte donation outcomes. Obstet Gynecol Surv 2016;71:301-6.

10. Li HW, Lee VC, Lau EY, Yeung WS, Ho PC, Ng EH. Role of baseline antral follicle count and anti-Mullerian hormone in prediction of cumulative live birth in the first in vitro fertilisation cycle: a retrospective cohort analysis. PLoS One 2013;8:e61095.

11. World Health Organization. WHO Laboratory Manual for the Examination of Human Semen and Sperm-cervical Mucus Interaction. 4th ed. Cambridge: Cambridge University Press; 1999.

12. World Health Organization. WHO Laboratory Manual for the Examination and Processing of Human Semen. 5th ed. Geneva: World Health Organization; 2010.

13. Begueria R, Garcia D, Obradors A, Poisot F, Vassena R, Vernaeve V. Paternal age and assisted reproductive outcomes in ICSI donor oocytes: is there an effect of older fathers? Hum Reprod 2014;29:2114-22.

14. Duran EH, Dowling-Lacey D, Bocca S, Stadtmauer L, Oehninger S. Impact of male age on the outcome of assisted reproductive technology cycles using donor oocytes. Reprod Biomed Online 2010;20:848-56.

15. Whitcomb BW, Turzanski-Fortner R, Richter KS, et al. Contribution of male age to outcomes in assisted reproductive technologies. Fertil Steril 2011;95:147-51.

16. Luna M, Finkler E, Barritt J, et al. Paternal age and assisted reproductive technology outcome in ovum recipients. Fertil Steril 2009;92:1772-5.

17. Kovac JR, Addai J, Smith RP, Coward RM, Lamb DJ, Lipshultz LI. The effects of advanced paternal age on fertility. Asian J Androl 2013;15:723-8.

18. Neaves WB, Johnson L, Petty CS. Age-related change in numbers of other interstitial cells in testes of adult men: evidence bearing on the fate of Leydig cells lost with increasing age. Biol Reprod 1985;33:259-69.

19. Johnson L, Zane RS, Petty CS, Neaves WB. Quantification of the human Sertoli cell population: its distribution, relation to germ cell numbers, and age related decline. Biol Reprod 1984;31:785-95.

20. Kuhnert B, Nieschlag E. Reproductive functions of the ageing male. Hum Reprod Update 2004;10;327-39.

21. Toriello HV, Meck JM, Professional Practice and Guidelines Committee. Statement on guidance for genetic counseling in advanced paternal age. Genet Med 2008;10:457-60.

22. American Society for Reproductive Medicine. Practice Committee guidelines. Sperm donation. Available from: https://www.asrm.org/Guidelines/. Accessed 25 Oct 2017.

23. British Andrology Society. Policy and guidelines. Sperm donation. Available from: http://www.britishandrology.org.uk/resources/policy-guidelines/. Accessed 25 Oct 2017.

24. Moskovtsev SI, Willis J, Mullen JB. Age-related decline in sperm deoxyribonucleic acid integrity in patients evaluated for male infertility. Fertil Steril 2006;85:496-9.

25. López G, Lafuente R, Checa MA, Carreras R, Brassesco M. Diagnostic value of sperm DNA fragmentation and sperm high-magnification for predicting outcome of assisted reproduction treatment. Asian J Androl 2013;15:790-4.

26. Singh NP, Muller CH, Berger RE. Effects of age on DNA double-strand breaks and apoptosis in human sperm. Fertil Steril 2003;80:1420-30.

27. Spanò M, Bonde JP, Hjøllund HI, Kolstad HA, Cordelli E, Leter G. Sperm chromatin damage impairs human fertility. The Danish First Pregnancy Planner Study Team. Fertil Steril 2000;73:43-50.

28. Sharma R, Agarwai A, Rohra VK, Assidi M, Abu-Elmagd M, Turki RF. Effects of increased paternal age on sperm quality, reproductive outcome and associated epigenetic risks to offspring. Reprod Biol Endocrinol 2015;13:35.

29. Frattarelli JL, Miller KA, Miller BT, Elkind-Hirsch K, Scott RT Jr. Male age negatively impacts embryo development and reproductive outcome in donor oocyte assisted reproductive technology cycles. Fertil Steril 2008;90:97-103.

30. Robertshaw I, Khoury J, Abdallah ME, Warikoo P, Hofmann GE. The effect of paternal age on outcome in assisted reproductive technology using the ovum donation model. Reprod Sci 2014;21:590-3.

31. Lambalk CB, Banga FR, Huirne JA, et al. GnRH antagonist versus long agonist protocols in IVF: a systematic review and meta-analysis accounting for patient type. Hum Reprod Update 2017;23:560-79.

32. Al-Inany HG, Youssef MA, Ayeleke RO, Brown J, Lam WS, Broekmans FJ. Gonadotrophin-releasing hormone antagonists for assisted reproductive technology. Cochrane Database Syst Rev 2016;(4):CD001750.

33. van Wely M, Kwan I, Burt AL, et al. Recombinant versus urinary gonadotrophin for ovarian stimulation in assisted reproductive technology cycles. Cochrane Database Syst Rev 2011;(2):CD005354.

34. Polyzos NP, Messini CI, Papanikolaou EG, et al. Vaginal progesterone gel for luteal phase support in IVF/ICSI cycles: a meta-analysis. Fertil Steril 2010;94:2083-7.

35. Malaspina D, Harlap S, Fennig S, et al. Advancing paternal age and the risk of schizophrenia. Arch Gen Psychiatry 2001;58:361-7.

36. Dalman C, Allebeck P. Paternal age and schizophrenia: further support for an association. Am J Psychiatry 2002;159:1591-2.

37. Reichenberg A, Gross R, Weiser M, et al. Advancing paternal age and autism. Arch Gen Psychiatry 2006;63:1026-32.

38. Durkin MS, Maenner MJ, Newschaffer CJ, et al. Advanced parental age and the risk of autism spectrum disorder. Am J Epidemiol 2008;168:1268-76.

39. Belloc S, Hazout A, Zini A, et al. How to overcome male infertility after 40: Influence of paternal age on fertility. Maturitas 2014;78:22-9.

40. Jennings MO, Owen RC, Keefe D, Kim ED. Management and counseling of the male with advanced paternal age. Fertil Steril 2017;107:324-8.