The effect of ageing on female fertility in an assisted reproduction programme in Hong Kong: retrospective study

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Objective. To analyse the effect of ageing on female fertility in an in vitro fertilisation programme in Hong Kong.
Design. Retrospective study.
Setting. University teaching hospital, Hong Kong.
Patients. Seven hundred and seventy-one women in whom 1336 cycles of in vitro fertilisation were initiated between 1 January 1986 and 31 December 1995.
Main outcome measures. Patient age and indications for treatment; hormonal response; and the number of cancelled cycles, oocytes retrieved, oocytes fertilised, cleaving embryos, embryos transferred, and clinical pregnancies.
Results. Compared with women aged ≤30 years (n=193), women aged ≥36 years (n=398) had a significantly higher cycle cancellation rate (19.3% versus 10.4%), fewer oocytes retrieved per retrieval cycle (6.6 versus 9.0), fewer oocytes fertilised per retrieval cycle (5.0 versus 7.0), fewer cleaving embryos per retrieval cycle (4.8 versus 6.8), and lower serum oestradiol level (9735 [standard deviation, 5681] pmol/L versus 10 708 [5916] pmol/L) despite a larger amount of human menopausal gonadotrophin having been used (all variables, P<0.01; Chi squared test). The clinical pregnancy rate per initiated cycle (range, 7.5%-13.0%) decreased with advancing age (P<0.01; Chi squared test).
Conclusion. Ageing has a significant deleterious effect on women's reproductive capability. Women should be encouraged to seek early medical advice and treatment for subfertility.

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Key words: Age factors; Female; Fertilization in vitro; Infertility/therapy; Ovulation induction; Pregnancy

Introduction

It is well recognised that the fertility of women decreases with age. The decline starts at 30 years of age and fertility becomes minimal by 45 years of age.[1] This phenomenon has been observed for both natural conception and assisted reproduction. A pioneering study of natural conception investigated a group of Hutterite women, who belonged to a Protestant sect that condemns the practice of contraception.[2] The mean age of the last confinement was 41 years and the interval between confinements increased with advancing maternal age, thus indicating the decline in fecundity with increasing age.[2]

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There is evidence that the chance of success of infertility treatment also decreases with advancing age.[3-10] In studies of artificial insemination, the cumulative pregnancy rates have been shown to decrease significantly as the ages of the women increase.[3-6] The pregnancy rates achieved by in vitro fertilisation (IVF) and gamete intrafallopian transfer have also been shown to decrease with increasing age.[7-10] There are no local data, however, on the effect of ageing on the outcome of assisted reproduction. In this study, we have analysed the IVF cycles initiated during a 10-year period to assess the effect of ageing on the success of IVF treatment in Hong Kong Chinese women.

Materials and methods

The records of the 1336 IVF cycles that were initiated in 771 women between 1 January 1986 and 31 December 1995 in the Department of Obstetrics and Gynaecology at the Queen Mary Hospital (QMH) were analysed. Up until late 1989, ovarian stimulation was achieved by using clomifene (clomiphene) citrate and

human menopausal gonadotrophins (HMGs). Thereafter, it was achieved by inducing downregulation with gonadotrophin-releasing hormone (GnRH) agonists and then by giving HMG (the 'long' protocol). A course of intranasal buserelin (Suprefact; Hoechst AG, Frankfurt, Germany) 150 μg four times daily was started on day 21 of the menstrual cycle preceding the treatment cycle. On day 2 of the treatment cycle, pelvic ultrasonography was performed and blood was taken to assess the 17-β-oestradiol (E_2) level. Treatment was started if no abnormality was detected by ultrasonography and when the E_2 level was <220 pmol/L. The regimen consisted of intramuscular HMG 150 IU and follicle-stimulating hormone (FSH) 150 IU on days 3 and 4. From day 5 onwards, intramuscular HMG was given at a dosage of 150 IU/d. The ovarian response was monitored by measuring the serum E_2 level and by performing pelvic ultrasonography. The dosage of HMG was increased if the ovarian response was unsatisfactory after at least 1 week of treatment. In patients with a history of poor ovarian response in previous treatment cycles, the 'short' protocol was used. In this protocol, buserelin therapy was started on day 2 of the treatment cycle.

Human chorionic gonadotrophin (HCG) 10 000 IU was given intramuscularly in the evening when the leading follicle was ≥18 mm in diameter, the serum E_2 level was >1100 pmol/L per leading follicle, and when there were at least three follicles with a diameter >15 mm. Treatment was discontinued if the ovarian response was poor or if a spontaneous surge in the luteinizing hormone (LH) level occurred. Oocytes were retrieved 36 to 38 hours after HCG injection and incubated for 4 to 6 hours before insemination. Up to four cleavage-stage embryos were transferred approximately 40 hours after insemination. Embryo transfer was cancelled if there was a failure in oocyte retrieval, fertilisation, or cleavage, or if there was an excessive risk of ovarian hyperstimulation syndrome.

The luteal phase of the cycle was supported by giving two booster doses of HCG (1500 IU) 5 days apart. If the serum E_2 level was >18 000 pmol/L before the administration of the ovulating dose of HCG or >7340 pmol/L during the luteal phase, or if there was clinical evidence of ovarian hyperstimulation, vaginal progesterone pessaries (Cyclogest; Cox Pharmaceuticals, Barnstaple, United Kingdom) 200 mg twice daily were given instead.

Patients were classified into the following three age-groups: ≤30 years, 31-35 years, and ≥36 years. The effect of ageing on the following factors was analysed: cycle cancellation rate, oocyte response, serum oestradiol level on the day of the administration of the ovulating dose of HCG, and the number of ampoules of HMG used per cycle. Clinical pregnancy was defined as either the detection of the presence of gestational sac(s) on ultrasound scans or histological evidence of gestational products. Success rates were expressed as the number of clinical pregnancies per initiated cycle, per oocyte retrieval cycle, and per embryo transfer cycle. Statistical analyses were performed using the Chi squared or Kruskal-Wallis test.

Results

The mean age of the 771 women studied was 33.7 years (standard deviation [SD], 3.1 years; range, 21-43 years). Of the 1336 IVF cycles, 193 (14.4%) were initiated in women aged 30 years or younger, 745 (55.8%) cycles in women aged 31 to 35 years, and 398 (29.8%) cycles in those aged 36 years and older. Table 1 shows the indications for IVF in the different age-groups. Tubo-peritoneal problems, pelvic endometriosis, and male-factor infertility constituted the major causes of subfertility among the couples involved. Table 2 shows the number of initiated and cancelled cycles, and the number of oocyte retrievals and embryo transfers. Of the 1336 initiated cycles, 184 (13.8%) were cancelled because of poor ovarian response or spontaneous LH surge. The cancellation rate varied significantly with age ($P<0.01$), rising from 10.4% in women aged ≤30 years to 19.3% in those aged ≤36 years. A total of 1152 cycles of oocyte retrieval (86.2% of initiated cycles)

Table 1. Indications for in vitro fertilisation

Indications	No. of cycles (%)			
	Age-group (years)			
	≤30, n=193	31-35, n=745	≥36, n=398	All ages, n=1336
Tubo-peritoneal	137 (71.0)	454 (60.9)	244 (61.3)	835 (62.5)
Pelvic endometriosis	18 (9.3)	85 (11.4)	60 (15.1)	163 (12.2)
Male-factor infertility	15 (7.8)	85 (11.4)	34 (8.5)	134 (10.0)
Idiopathic	13 (6.7)	72 (9.7)	32 (8.0)	117 (8.8)
Tubal + male-factor infertility	5 (2.6)	13 (1.7)	19 (4.8)	37 (2.8)
Others	5 (2.6)	36 (4.8)	9 (2.3)	50 (3.7)

Table 2. Details of in vitro fertilisation cycles

Cycles	Age-group (years)				P value*
	≤30	31-35	≥36	All ages	
No. of initiated cycles	193	745	398	1336	
No. of cancelled cycles (% of initiated cycles)	20 (10.4%)	87 (11.7%)	77 (19.3%)	184 (13.8%)	<0.01
No. of oocyte retrievals (% of initiated cycles)	173 (89.6%)	658 (88.3%)	321 (80.7%)	1152 (86.2%)	<0.01
No. of embryo transfers (% of oocyte retrieval cycles)	154 (89.0%)	574 (87.2%)	275 (85.7%)	1003 (87.1%)	<0.01

* P values comparing age-groups ≤30 and ≥36 years (χ^2 test)

Table 3. Ovarian responses during in vitro fertilisation cycles

	Age-group (years)				P value*
	≤30	31-35	≥36	All ages	
No. of follicles aspirated (No. per oocyte retrieval cycle)	2219 (12.8)	7752 (11.8)	2921 (9.1)	12 892 (11.2)	<0.01
No. of oocytes retrieved (No. per oocyte retrieval cycle)	1560 (9.0)	5468 (8.3)	2108 (6.6)	9136 (7.9)	<0.01
No. of oocytes fertilized (No. per oocyte retrieval cycle)	1213 (7.0)	4117 (6.3)	1594 (5.0)	6924 (6.0)	<0.01
Fertilisation rate	78.4%	75.4%	74.1%	75.5%	0.37
No. of fertilized oocytes cleaved (No. per oocyte retrieval cycle)	1173 (6.8)	4024 (6.1)	1549 (4.8)	6746 (5.9)	<0.01
Cleavage rate	95.9%	97.4%	96.6%	96.9%	1.00
No. of embryos transferred (No. per embryo transfer cycle)	461 (3.0)	1656 (2.9)	843 (3.1)	2960 (3.0)	

* P values comparing age-groups ≤30 and ≥36 years (χ^2 test)

Table 4. Hormonal response during in vitro fertilisation

	Age-group (years)				P value*
	≤30	31-35	≥36	All ages	
Serum oestradiol level on day of HCG† treatment (Mean [SD]) (pmol/L)	10 708 (5916)	10 176 (5865)	8306 (4865)	9735 (5681)	<0.01
No. of ampoules of HMG‡ used per initiated cycle	28.2	30.9	36.3	32.1	<0.01

* P values comparing age-groups ≤30 and ≥36 years (χ^2 test)
† HCG human chorionic gonadotrophin
‡ HMG human menopausal gonadotrophin

were performed, and 1003 (87.1%) of these cycles involved subsequent embryo replacement.

The ovarian responses, arranged according to age-group, are shown in Table 3. Advancing age had a significantly adverse effect on the number of follicles aspirated (P<0.01), the number of oocytes retrieved (P<0.01), the number of oocytes fertilised (P<0.01), and the number of cleaving fertilised oocytes (P<0.01). The overall fertilisation rate was 75.5%, and the rate did not differ significantly among the three age-groups (P=0.37). The overall cleavage rate was 96.9% and was not affected by age (P=1.00).

The number of ampoules of HMG used in the initiated cycles increased significantly with advancing age (P<0.01) [Table 4]. The average number of ampoules used per initiated cycle increased from 28.2 in women aged ≤30 years to 36.3 in those aged ≥36 years. Furthermore, the serum E_2 level achieved just before the administration of the ovulating dose of HCG decreased significantly with advancing age (P<0.01), dropping from 10 708 (SD, 5916) pmol/L in women aged ≤30 years to 8306 (4865) pmol/L in those aged ≥36 years (Table 4).

There were a total of 146 clinical pregnancies. The overall clinical pregnancy rate per initiated cycle was 10.9% and the rate decreased significantly with advancing age (P<0.05) [Table 5]. The clinical pregnancy rates per oocyte retrieval cycle and per embryo transfer cycle also showed a falling trend with increasing age of the women. However, the differences were not statistically significant (Table 5).

Table 5. Clinical pregnancy rates

	Age-group (years)				P value*
	≤30	31-35	≥36	All ages	
No. of clinical pregnancies	25	91	30	146	
Clinical pregnancy rate (%)					
per initiated cycle	13.0	12.2	7.5	10.9	0.03
per oocyte retrieval cycle	14.5	13.8	9.3	12.7	0.11
per embryo transfer cycle	16.2	15.9	10.9	14.6	0.13

* P values comparing age-groups ≤30 and ≥36 years (χ^2 test)

Discussion

The results of this retrospective analysis show that the cancellation rate (19.3%) for the women aged 36 years and older in the IVF programme at the QMH was almost double that for the women aged 30 years or younger (10.4%). Because the long buserelin protocol was used in most of the ovarian stimulation cycles, spontaneous LH surges were rare and most of the cancellations were caused by poor ovarian response. In those who underwent oocyte retrieval, the number of oocytes obtained and the peak serum E_2 levels were significantly lower in the older women, despite more ampoules of HMG being used in these women. Hence, older women had a poorer ovarian response to gonadotrophin stimulation. Possible mechanisms underlying this poor ovarian response include progressive follicular depletion and declining follicular competence with advancing age, which correlate with the decrease in the level of inhibin and increase in the level of FSH in the later reproductive years.[11,12]

The fertilisation and embryo cleavage rates were similar among the three age-groups. Because the number of oocytes obtained per cycle decreased significantly with increasing age, the number of embryos obtained per cycle (range, 4.8-6.8) also decreased significantly. In the IVF programme at the QMH, a maximum of four embryos is replaced. Consequently, there was no difference in the number of embryos replaced among the three age-groups. However, the clinical pregnancy rates per cycle of oocyte retrieval and per cycle of embryo transfer showed a decreasing trend with advancing age, although this result was not statistically significant for this small sample. The satisfactory pregnancy rates and lower miscarriage rates that have been achieved in older women receiving oocytes from young donors have led to the postulation that female reproductive ageing is due to declining oocyte quality.[1,13-15] Whereas some studies of oocyte donation have found lower pregnancy rates in the older recipients compared with the younger recipients,[16-18] other studies have shown that this effect can be corrected by increasing the doses of oestrogen or progesterone in the recipients' stimulation protocols.[19,20] It is thus likely that the age-related decline in fecundity is primarily a result of the ageing oocytes, rather than a reduction in endometrial receptivity, which may be a contributing factor but is likely to be remediable by the hormone stimulation provided in the stimulation protocols.

The dosage of gonadotrophins used in the IVF programme at the QMH is adjusted according to the ovarian response. A higher initial dose is not used in the cycles for older women, because not all women older than 35 years have poor ovarian response, and excessive ovarian stimulation by gonadotrophins can result in ovarian hyperstimulation syndrome. Factors other than the age of the woman might predict poor ovarian response in IVF treatment; they include a family history of premature ovarian failure, a history of pelvic surgery on the adnexa, endometriosis, obesity, idiopathic infertility, and smoking.[21] Chan et al[22] have shown that high basal FSH levels are associated with a worse response to ovarian stimulation in IVF treatment. There is also evidence that a low level of plasma inhibin[23,24] and a relatively high level of early follicular phase E_2[25-27] are associated with a poor ovarian response. The clomifene test, which measures the ovary's ability to control FSH secretion, helps to predict which women would respond well to stimulation and which would respond badly.[21] Chang et al[28] have recently suggested that there is an age-related decrease in the number of antral follicles. Hence, measuring the number of antral follicles might be helpful in predicting a patient's response to ovarian stimulation.

Various therapeutic strategies have been tried to improve the success of patients who have poor response to the conventional long protocols of ovarian stimulation. The use of the short GnRH protocol—that is, simultaneously giving an GnRH analogue and gonadotrophins—takes advantage of the initial hormonal 'flare-up' produced by the analogues. Conversely, reducing the dose of the GnRH analogue, with the aim of reducing the intensity of pituitary suppression,

has been reported to result in lower cycle cancellation rates and better ovarian responses.[29] Preliminary data have also shown that using recombinant gonadotrophins may result in the production of more oocytes and more embryos compared with when gonadotrophins derived from urinary extraction is used.[30,31] Further studies are necessary to show whether these drugs can achieve better responses in poor responders. Oocyte donation from young women is a valid alternative for women who have poor ovarian reserve.

In modern society, women tend to defer marriage and postpone child-bearing. This trend, together with the fact that fertility decreases with advancing age, have posed challenges to doctors involved in the treatment of infertility. Women should be educated and informed of the decline in fertility with ageing and the comparatively low cost-effectiveness of assisted reproduction treatment in the older age-groups. They should be encouraged to promptly seek medical advice for subfertility, so that investigations can be performed and treatment given at an early stage. The option of receiving donated oocytes to improve the success of IVF in older women should also be explained.

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