Article

Minimal ovarian stimulation with clomiphene citrate: a large-scale retrospective study

Born in 1957 in Japan, Shokichi Teramoto graduated in 1990 from Kanazawa University School of Medical Science, Ishikawa, Japan. Subsequently he trained in the Department of Obstetrics and Gynecology at the Graduate School of the same university. From 1994 he trained as an infertility specialist under the guidance of Dr Osamu Kato, in 1996 becoming Vice Director of Kato Ladies Clinic, an IVF center in Tokyo. Since then, he has devoted himself to establishing an ovulation stimulation method using clomiphene citrate. Other interests include obtaining oocytes from leukaemia patients and building an oocyte retrieval–cryopreservation system for leukaemia patients.

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Abstract

Enclomiphene, an isomeric component of clomiphene citrate, acts antagonistically to the oestradiol receptor at the hypothalamus level, inhibiting both negative and positive feedback, and resulting in the induction of ovarian stimulation and suppression of ovulation. The minimal ovarian stimulation protocol takes full advantage of these characteristics of clomiphene citrate. Administration of 50 mg clomiphene citrate is initiated on cycle day 3, and from day 8 patients receive 150 IU of FSH every other day. When the size of the dominant follicle and the oestradiol concentration reach the predefined values, gonadotrophin-releasing hormone agonist is administered to induce follicular maturation. Oocytes are then retrieved 32–35 h later. Because the short half-life of enclomiphene (24 h) is of critical importance in this protocol, it is necessary to continue oral administration of clomiphene citrate until the day before maturation is triggered. Of all 43,433 cycles initiated, the rates for oocyte retrieval and embryo cleavage were 83 and 64% respectively. The mean number of oocytes retrieved was 2.2. The rates for live births, miscarriages, and ectopic pregnancies, in relation to initiated cycles, including cases of frozen–thawed transfer, were 11.1, 3.4 and 0.2% respectively.

Keywords: *enclomiphene*, *GnRH agonist*, *half-life*, *oral contraception*

Introduction

The aim of this retrospective study was to evaluate the efficacy of the minimal ovarian stimulation method with the use of clomiphene citrate. Ovarian induction involves the challenge of finding a way to control ovulation inhibition. In the early days, much effort was made to achieve this by detecting the onset of the LH surge by frequently measuring the LH concentration in the urine (Edwards *et al*., 1980). Next, the gonadotrophin-releasing hormone (GnRH) agonist long/short protocol was developed, utilizing down-regulation, and this has become the mainstream procedure in IVF until the present day. However, shortcomings have also been pointed out, including the flare-up action of LH and its continued influence after use has been terminated. GnRH antagonist was developed against this background, and seemed to have solved the problems relating to GnRH agonist. However, new concerns over its adverse effect on follicular development have been raised, suggesting that the combined use of recombinant FSH and GnRH antagonist will lead to a low LH environment. Such ovarian induction methods based on drugs that inhibit the pituitary function presuppose the use of large amounts of human menopausal gonadotrophin (HMG) or FSH in combination. It can be said, therefore, that the issue of their influence on folliculogenesis and steroidogenesis has been left unquestioned. Naturally, the cost of IVF has been on the increase, limiting the number of patients who can enjoy the benefits of such treatment and causing financial assistance for such treatment to place an increasing burden on the public purse. Under such circumstances, Edwards *et al*. (1996) pressed the need for a change in conventional ovarian induction methods, raising questions concerning such methods in 1995. The present study is one such answer to this issue, devised as part of an effort to respond to this question carried out in the Far East.

Materials and methods

A total of 44,345 cycles from women who had given their consent to ovarian stimulation for the purpose of oocyte retrieval for IVF–embryo transfer from January 2001 to December 2005 were studied. The patient's background, including mean age, day 3 oestradiol/FSH concentrations, the mean ± SD values for body mass index (BMI) and menstrual cycles, are shown in **Table 1**. Ovarian stimulation was carried out using clomiphene citrate (Serophene; Serono Laboratories, Japan) in combination with a minimum amount of urinary HMG (Humegon 150; Organon, Japan) or recombinant FSH (Follistim 150 and Gonal F; Organon and Serono respectively) (**Figure 1**). Administration of clomiphene citrate was initiated from day 3 at 50 mg/day and was continued until the day before GnRH agonist (GnRHa) was given as the maturation trigger. Maturation was triggered when the diameter of the dominant follicle had reached 18 mm or more and also the oestradiol concentration per follicle had reached 300 pg/ml or more (**Table 2**). In cases where several follicles showed promising development, this was confirmed by ultrasonography performed on day 8, and 150 IU of urinary HMG or recombinant FSH was then administered every other day until the day before maturation was triggered. Maturation was triggered using 300 μg of GnRHa nasal spray (Suprecur nasal solution 0.15%; Sanofi Aventis, USA), and the oocytes were retrieved after 32–35 h. In cases where initiation of the LH surge was suspected, however, oocytes were retrieved 28–30 h after the GnRHa nasal spray was given (**Table 2**). Luteal phase support was provided by administering 30 mg/day of dydrogesterone (DUP, Duphastone; Solvay Pharmaceuticals, USA). The progesterone concentration was measured on day 0 and days 6 or 8 (mean value), and day 12 of embryo transfer for 4-cell-stage transfer. The same was measured on day 0, and days 3 or 5 (mean value) and day 7 for blastocyst transfers. In cases where progesterone concentration measured for the mean value was falling, 125 mg of progesterone deposit (Proge depot; Mochida Pharmaceutical Company Limited) was administered according to the case. The β-human chorionic gonadotrophin (βHCG) concentration was measured on day 12 of 4-cell-stage transfer or on day 7 of blastocyst transfer to determine whether implantation had been successful or not. The final success rate of the transfer was based on the live births.

Results

The oestradiol concentration per developed follicle decreased as the number of developed follicles increased, from 394 pg/ml in the case of one follicle developed, to 197 pg/ml, in the case of eight developed follicles. The oestradiol concentration per retrieved oocytes decreased from 578 pg/ml, in the case of one follicle retrieved, to 268 pg/ml, in the case of eight follicles retrieved (**Table 3**). The oestradiol concentration on the day when follicular maturation was triggered in the youngest age group was 1000 pg/ml, which was the equivalent to having three developed follicles. This concentration decreased in the most advanced age group to 700 pg/ml, which was equivalent to having two developed follicles. The LH concentration and the required duration for ovarian stimulation were not influenced by the age factor (**Table 4**). Emergency oocyte retrieval due to the onset of the LH surge was required in 3.5% and oocyte retrieval just before ovulation due to completion of the LH surge was required in 1.6% of all cycles. In either case, the ovulation rate was between 2 and 3%, demonstrating the efficacy of the clomiphene cycle in avoiding ovulation (**Table 5**). The rates for oocyte retrieval, fertilization, and cleavage of embryo were 93, 84 and 71% respectively in the youngest age group, and decreased to 71, 57 and 48% respectively in the most advanced age group (**Table 6**). The number of follicles developed and the number of embryos cleaved were 5.3 and 1.9 respectively in the youngest age group, and decreased as the age advanced to 1.5 and 0.7 respectively in the most advanced age group (**Table 6**). The rate of cycles in which HMG was required for ovarian stimulation, and the mean dose, were 94% and 337 IU respectively in the youngest age group, and both were decreased with advancing age, to 55% and 167 IU in the oldest age group (**Table 4**). The administration of oral contraceptives in the previous cycle improved both the number of oocytes retrieved and number of embryos cleaved in all age groups, except that of 45 years old and above (**Table 7** and **Figure 2**). The number of embryos transferred was very low, 1.6 at the most (**Table 6**), although it varied according to age and year. The live birth rate for the transfer of fresh 4-cell-stage embryos was highest in the youngest age group (14.6%) and decreased as age advanced, to lower than 5% at around 40 years old (**Table 8**). The live birth rate for frozen–thawed embryo transfer per transfer cycle was higher than that for fresh embryo transfer in both 4-cell-stage embryo transfer and blastocyst transfer, at 40–50% for 38 years old and younger (**Table 8**). The live birth rate per oocyte retrieval cycle was 28% in the youngest age group. This rate, however, dramatically decreased at age 42 years old or above (**Table 9**).

A significant difference ($P < 0.0001$) in LH concentration was observed between the two groups: the group where the oestradiol concentration on the day of maturation triggering was less than 500 pg/ml, compared with the group with an oestradiol concentration of 500 pg/ml or above and less than 1000 pg/ml. However, in the group with an oestradiol concentration of 1000 pg/ml or above, the LH concentration was stable in the range of 7–8 IU/l and showed no significant variation between groups. This was closely related to the fact that the LH concentration became lower with higher patient age, suggesting that the premature LH surge is strongly inhibited, regardless of the oestradiol concentration, in the age bracket with a normal LH base (**Table 10**). There were two major cases of fetal anomalies, namely a case of cardiac anomaly (ventricular septal defect) and a case of Down's syndrome.

The course of the progesterone concentration differed depending on the occurrence of implantation. Where implantation occurred at the normal time, the progesterone concentration increased along with the increase in the β-HCG concentration. However, when implantation did not take place, the concentration began to decrease 10 days after ovulation (**Table 11**).

Table 1. Background of patients.

Age (years)	*27–29*	*30–32*	*33–35*	*36–38*	*39–41*	*42–44*	*45–47*	*Total*
No. of initiated cycles	1072	3335	6286	8465	10688	9732	4767	44,345
No. of patients	581	1583	2572	2719	2425	1396	463	11,739
Day 3 oestradiol pg/ml: mean (SD)	43 (19)	47 (19)	46 (18)	47 (19)	47 (19)	47 (22)	45 (22)	47 (20)
Day 3 FSH IU/l: mean (SD)	10.1 (3.3)	11.0 (4.2)	11.2 (5.0)	12.3 (5.4)	13.7 (5.9)	14.3 (6.0)	16.0 (6.3)	13.0 (5.8)
BMI: mean (SD)	20.9 (2.7)	20.6 (3.3)	20.2 (2.4)	20.3 (2.3)	20.7 (2.5)	21.0 (2.8)	21.2 (2.8)	20.7 (2.8)
BMI: range	14.8–41.4	16.0–37.3	16.0–28.7	15.9–30.7	14.8–35.2	15.4–41.4	15.4–32.8	16.4–33.1
Menstrual cycle: mean (SD)	32.4 (5.0)	31.7 (4.8)	30.7 (4.4)	30.5 (4.8)	29.7 (3.5)	29.6 (4.1)	29.7 (6.2)	30.4 (4.5)
Menstrual cycle: range (days)	24–50	22–60	22–60	24–60	22–50	23–50	21–60	21–60
No. of past embryo transfers: mean (SD)	0.9 (1.4)	1.2 (1.7)	1.5 (1.9)	2.1 (2.7)	2.9 (3.2)	4.0 (4.4)	5.5 (6.1)	2.9 (3.8)

There were significant differences between each age bracket in day 3 (D3) FSH, body mass index (BMI), menstrual cycle, and the number of past embryo transfers, $P < 0.0001$ (analysed by ANOVA).

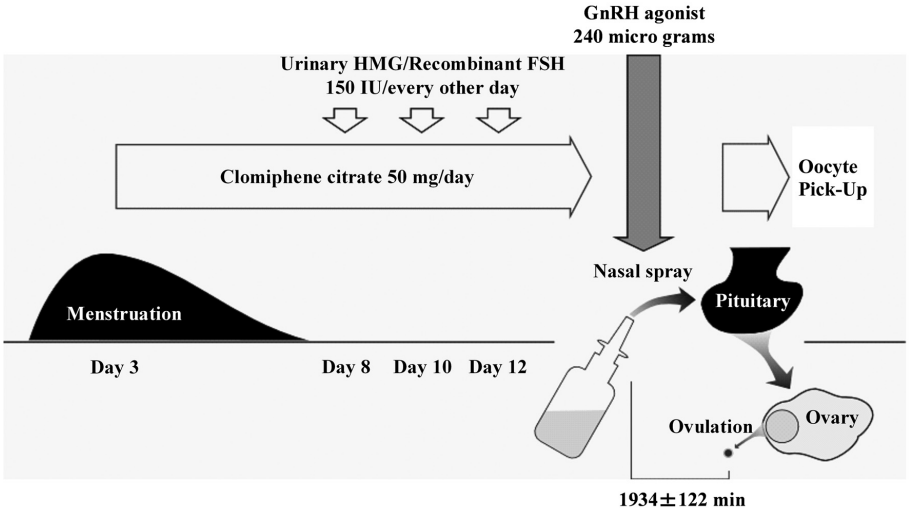

Figure 1. Clomiphene cycle protocol. Administration of clomiphene citrate was initiated on day 3 and continued until the day before maturation triggering. Administration of HMG or FSH was initiated on day 8 at 150 IU per session and given to the patient every other day. The final maturation triggering was induced by administering 240 μg GnRHa nasal spray. The mean time until oocyte retrieval was 1934 min.

Table 2. Timing of gonadotrophin-releasing hormone agonist (GnRHa) administration and the measures for addressing the increase in the LH concentration.

LH concentration/ LH basal concentration	*Findings*	*Measures to take*
<1.5	Diameter of the dominant follicle ≥18 mm; oestradiol concentration per follicle of 18 mm in diameter ≥300 pg/ml; oestradiol concentration per follicle of 13 mm in diameter ≥150 pg/ml	Administer GnRHa nasal spray at 2400, and retrieve oocytes in 32–35 h (normal oocyte retrieval)
1.5–3	–	Immediately administer GnRHa nasal spray and retrieve oocytes in 30 h (emergency oocyte retrieval)
3–5	–	Immediately administer GnRHa nasal spray and retrieve oocytes in 28 h (emergency oocyte retrieval).
>5	Re-examine in 4 h; before the LH surge peak: normally increase by two-fold	Retrieve oocytes in 16–20 h without administering GnRHa nasal spray (surge oocyte retrieval)
	Re-examine in 4 h; after the LH surge peak: both the oestradiol and LH concentrations decrease	Immediately retrieve oocytes (surge oocyte retrieval)

Table 3. Relationship between the oestradiol concentration and the number of follicles matured.

No. of follicles matured or oocytes retrieved	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*
Oestradiol per matured follicle: mean (SD) (pg/ml)	394 (164)	311 (113)	269 (90)	246 (81)	230 (196)	217 (66)	206 (64)	197 (62)
No. of cycles in which oocytes were matured	7419	7622	5296	2885	1470	751	476	291
Oestradiol per retrieved oocyte: mean (SD) (pg/ml)	578 (297)	399 (165)	333 (134)	306 (119)	292 (91)	282 (112)	268 (100)	268 (86)
No. of cycles in which oocytes were retrieved	9790	6307	2889	1231	580	292	165	108

Criteria for mature follicles: diameter of the dominant follicle ≥18mm, the other follicle ≥14 mm.

Table 4. Oestradiol and LH concentrations on the day on which follicular maturation was triggered and the rate of cycles to which HMG was used for ovarian stimulation and their mean dose.

Age (years)	*27–29*	*30–32*	*33–35*	*36–38*	*39–41*	*42–44*	*45–47*	*Total*
Oestradiol: mean (SD) (pg/ml)	961 (631)	974 (644)	914 (520)	891 (455)	868 (450)	767 (367)	694 (320)	850 (465)
LH: mean (SD) (IU/l)	8.2 (3.6)	7.8 (3.1)	7.7 (3.4)	7.7 (3.2)	7.7 (3.4)	8.1 (3.5)	8.6 (4.3)	7.9 (3.5)
Days until maturation triggering: mean (SD)	15.0 (2.3)	14.7 (2.4)	14.5 (2.3)	14.3 (2.6)	14.4 (2.9)	14.5 (3.3)	14.8 (3.8)	14.5 (2.9)
HMG/FSH usage rate (%)	94.4	91.6	90.1	85.9	80.9	70.0	55.7	82.4
HMG/FSH dose (IU)	337	354	336	300	265	218	167	257

There were significant differences between each age bracket in both categories, $P < 0.0001$ (analysed by ANOVA).
There were significant differences between each age bracket in the dose category, $P < 0.0001$ (analysed by ANOVA).
In cases where there was only one developed follicle or the rate of the development was too rapid, HMG was not administered.

Table 5. Analysis of failed oocyte retrieval and post-ovulation rates for normal, emergency and post-LH surge oocyte retrieval.

Age (years)	*27–29*	*30–32*	*33–35*	*36–38*	*39–41*	*42–44*	*45–47*	*Total*
Normal oocyte retrieval after GnRHa	992	3138	5649	7933	10,001	8991	4335	41,039
No oocyte (%)	53 (5.3)	202 (6.4)	463 (7.8)	800 (10.1)	1463 (14.6)	1634 (18.2)	1052 (24.3)	5667 (13.8)
Post-ovulation (%)	14 (1.4)	36 (1.1)	74 (1.2)	105 (1.3)	138 (1.4)	156 (1.7)	100 (2.3)	623 (1.5)
Emergency oocyte retrieval after GnRHa	21	40	101	240	362	475	296	1535
No oocyte (%)	2 (9.5)	5 (12.5)	17 (16.8)	61 (25.4)	111 (30.7)	151 (31.8)	121 (40.9)	468 (30.5)
Post-ovulation (%)	0 (0.0)	2 (5.0)	1 (1.0)	5 (2.1)	6 (1.7)	6 (1.3)	10 (3.4)	30 (2.0)
Oocyte retrieval after LH surge	23	44	71	111	139	168	130	686
No oocyte (%)	1 (4.3)	11 (25.0)	20 (28.2)	41 (36.9)	50 (36.0)	64 (38.1)	73 (56.2)	260 (37.9)
Post-ovulation (%)	0 (0.0)	1 (2.3)	2 (2.8)	4 (3.6)	2 (1.4)	4 (2.4)	4 (3.1)	17 (2.5)

Emergency oocyte retrieval: cases where oocytes were retrieved 28–30 h after the nasal administration of gonadotrophin-releasing hormone (GnRH) agonist due to the onset of the premature LH surge.
Oocyte retrieval after LH surge: cases where oocytes were retrieved without the administration of GnRHa because the LH surge had been either completed or was too advanced.

Table 6. Outcome of embryo culture in relation to initiated cycles (excludes cancelled cycles due to spontaneous ovulation), mean numbers of follicles matured, and oocytes retrieved, fertilized and cleaved and the mean number of transferred embryos.

Age (years)	*27–29*	*30–32*	*33–35*	*36–38*	*39–41*	*42–44*	*45–47*	*Total*
No. of initiated cycles	1031	3207	6117	8258	10,486	9598	4736	43,433
No. of retrieved cycles (%)	958 (92.9)	2944 (91.8)	5517 (90.2)	7215 (87.4)	8676 (82.7)	7522 (78.4)	3340 (70.5)	36,172 (83.3)
No. of fertilized cycles (%)	866 (84.0)	2666 (83.1)	5008 (81.9)	6437 (77.9)	7583 (72.3)	6444 (67.1)	2685 (56.7)	31,689 (73.0)
No. of cleaved cycles (%)	727 (70.5)	2325 (72.5)	4445 (72.7)	5728 (69.4)	6657 (63.5)	5682 (59.2)	2270 (47.9)	27,834 (64.1)
Mean no. of matured follicles[a,b]	5.30	4.20	3.44	2.79	2.25	1.75	1.53	2.52
Mean no. of retrieved oocytes[a,b]	3.96	3.53	3.01	2.43	1.98	1.59	1.21	2.20
Mean no. of fertilized oocytes[a,b]	2.74	2.48	2.18	1.78	1.46	1.18	0.88	1.60
Mean no. of cleaved oocytes[a,b]	1.88	1.82	1.69	1.43	1.18	0.97	0.71	1.26
Mean no. of transferred embryos in fresh day 2 transfers (SD)	1.62 (0.64)	1.53 (0.59)	1.50 (0.57)	1.49 (0.60)	1.51 (0.68)	1.47 (0.70)	1.47 (0.65)	1.49 (0.64)
Mean no. of transferred embryos in frozen–thawed 4-cell-stage transfers (SD)	1.21 (0.42)	1.23 (0.43)	1.12 (0.36)	1.06 (0.23)	1.13 (0.43)	1.18 (0.45)	1.27 (0.67)	1.14 (0.40)
Mean no. of transferred embryos in frozen–thawed blastocyst transfers (SD)	1.07 (0.28)	1.04 (0.22)	1.02 (0.15)	1.03 (0.16)	1.04 (0.21)	1.05 (0.22)	1.05 (0.23)	1.03 (0.19)

% = rate in relation to initiated cycles.
[a]There were significant differences between each age bracket in each category, $P < 0.001$ (analysed by ANOVA).
[b]The population of each mean value is the number of initiated cycles.

Table 7. Influence of oral contraceptives (OC) given in the previous cycle.

Age (years)	*27–29*	*30–32*	*33–35*	*36–38*	*39–41*	*42–44*	*45–47*
No OC given							
No. of retrieved oocytes	2.55	1.96	2.27	1.93	1.64	1.24	1.26
No. of cleaved oocytes	1.45	1.48	1.68	1.70	1.36	1.09	1.18
OC given to previous single cycle							
No. of retrieved oocytes	2.89	2.67	2.48	1.91	1.71	1.46	1.19
No. of cleaved oocytes	1.81	1.61	1.56	1.21	1.12	0.93	0.74
OC given to previous two consecutive cycles							
No. of retrieved oocytes	4.02	3.88	3.46	2.67	1.96	1.52	0.68
No. of cleaved oocytes	2.66	2.37	2.15	1.73	1.27	0.92	0.35

OC given to previous single cycle: oral contraceptives were only administered during the luteal phase immediately before the IVF cycle.
OC given to previous two consecutive cycles: OC were administered to the immediate two previous cycles. Mainly Marvelon® (Organon) was used but Planovar® (Wyeth, USA) was also occasionally used; see **Figure 2**.
There were significant differences between each category (no OC given, OC given to previous single cycle, and OC given to previous two consecutive cycles) in all the age brackets, $P < 0.0001$ (analysed by ANOVA).

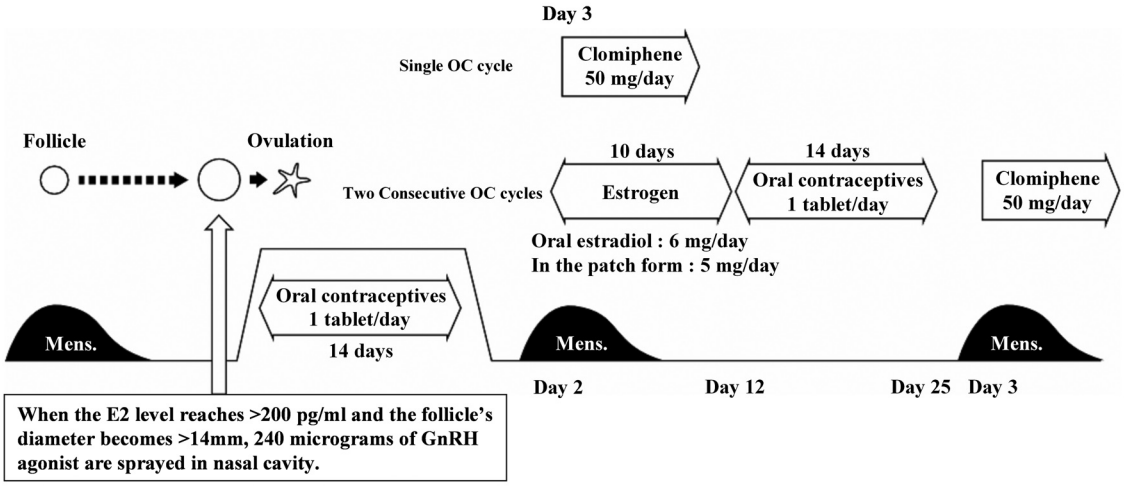

Figure 2. Method of oral contraceptive administration. Single OC cycle: The luteal phase of the cycle immediately before the IVF cycle is induced by a nasal spray of GnRHa, and OC (mainly Marvelon®; Organon) is administered for 14 days from the day after the GnRHa administration. Two consecutive OC cycles: in addition to the treatment for the single OC cycle, oestrogen (oral oestradiol 6 mg/day or conjugated oestrogen 3.75 mg/day) is administered from day 6 after completion of OC administration (= day 2) for 10 days until day 12. Then, OC is administered again for 14 days from day 12 to day 25. Setting day 7 of the final termination day of the OC administration as day 3, administration of clomiphene citrate is initiated.

Table 8. Outcome of fresh 4-cell-stage embryo transfers, frozen–thawed 4-cell-stage embryo transfers and blastocyst transfer.

Age (years)	*27–29*	*30–32*	*33–35*	*36–38*	*39–41*	*42–44*	*45–47*	*Total*
No. of initiated cycles	1072	3335	6286	8465	10,688	9732	4767	44,345
Fresh day 2 embryo transfers	499	1460	2671	3279	3447	2522	1011	14,889
Live births (%)	156 (14.6)	450 (13.5)	661 (10.5)	626 (7.4)	336 (3.1)	93 (1.0)	4 (0.1)	2326 (5.2)
Miscarriages (%)	22 (2.1)	75 (2.2)	157 (2.5)	208 (2.5)	215 (2.0)	88 (0.9)	18 (0.4)	783 (1.8)
Ectopic pregnancies (%)	3 (0.3)	14 (0.4)	25 (0.4)	23 (0.3)	11 (0.1)	7 (0.1)	0 (0.0)	83 (0.2)
Frozen–thawed 4-cell stage embryo transfers	19	56	121	125	114	79	26	540
Live births (%)	7 (0.7)	17 (0.5)	30 (0.5)	26 (0.3)	18 (0.2)	4 (0.0)	0 (0.0)	102 (0.2)
Miscarriages (%)	2 (0.2)	6 (0.2)	16 (0.3)	17 (0.2)	13 (0.1)	5 (0.1)	0 (0.0)	59 (0.1)
Ectopic pregnancies (%)	0 (0.0)	2 (0.1)	1 (0.0)	2 (0.0)	1 (0.0)	0 (0.0)	0 (0.0)	6 (0.0)
Frozen–thawed blastocyst transfers	257	863	1507	1561	1291	430	47	5946
Live births (%)	138 (12.9)	454 (13.6)	757 (12.0)	652 (7.7)	403 (3.8)	87 (0.9)	4 (0.1)	2495 (5.6)
Miscarriages (%)	24 (2.2)	81 (2.4)	164 (2.6)	184 (2.2)	167 (1.6)	58 (0.6)	7 (0.1)	685 (1.5)
Ectopic pregnancies (%)	1 (0.1)	1 (0.0)	2 (0.0)	4 (0.0)	2 (0.0)	0 (0.0)	0 (0.0)	10 (0.0)

% = rate in relation to the number of initiated cycles (population).

Table 9. Outcome of embryo transfers: the accumulated rates for pregnancies, miscarriages, and ectopic pregnancies in relation to the total number of oocyte retrieval cycles, including the cases of frozen–thawed embryo transfers.

Age (years)	*27–29*	*30–32*	*33–35*	*36–38*	*39–41*	*42–44*	*45–47*	*Total*
No. of oocyte retrieval cycles	1072	3335	6286	8465	10,688	9732	4767	44,345
No. of embryo transfer cycles (%)	775 (72.3)	2379 (71.3)	4299 (68.4)	4965 (58.7)	4842 (45.3)	3031 (31.1)	1084 (22.7)	21,375 (48.2)
No. of patients receiving embryo transfers[a]	651	1971	3491	4100	4087	2764	1062	18,126
Live births (%)	301 (28.1)	921 (27.6)	1448 (23.0)	1304 (15.4)	757 (7.1)	184 (1.9)	8 (0.2)	4923 (11.1)
Miscarriages (%)	48 (4.5)	162 (4.9)	337 (5.4)	409 (4.8)	395 (3.7)	151 (1.6)	25 (0.5)	1527 (3.4)
Ectopic pregnancies (%)	4 (0.4)	17 (0.5)	28 (0.4)	29 (0.3)	14 (0.1)	7 (0.1)	0 (0.0)	99 (0.2)
Pregnancies (%)	353 (32.9)	1100 (33.0)	1813 (28.8)	1742 (20.6)	1166 (10.9)	342 (3.5)	33 (0.7)	6549 (14.8)
Rate of patients obtaining a live newborn baby (%)[b]	51.8	58.2	55.3	48.0	31.2	13.2	1.7	41.9

% = rate in relation to initiated cycles.
[a]Embryo transfer cycles here includes supernumerary embryo transfers.
[b]This figure shows the rate at which patients who received IVF obtained a live newborn baby during the period covered by this study (2001–2005).

Table 10. Relationship between oestradiol and LH concentrations on the day of the maturation triggering for patients who underwent oocyte retrieval from 2004 to 2005.

Oestradiol range pg/ml	*≤500*	*500–1000*	*1000–1500*	*1500–2000*	*2000–2500*	*Total (mean oestradiol: 764)*
No. of cycles	2328	3560	1296	358	104	7733
Mean LH IU/l	10.7[a]	8.2[a]	7.6[b]	7.5[b]	7.9[b]	8.8
% of patients where LH ≥15 IU/l	21.5	5.2	2.5	2.2	2.9	9.8
Mean age	41.0	39.3	38.1	37.1	35.6	39.5
% of patients aged ≥40 years	68.8	52.8	41.0	26.8	18.3	54.1

[a]There was a significant difference between these groups, analysed by *t*-test, $P < 0.0001$.
[b]There was no significant difference between these groups (analysed by ANOVA).
When the LH concentration was 15 IU/l (≈mean + 2SD) or above, this was ascertained as the initiation of the LH surge.

Table 11. The shift in the progesterone concentration after embryo transfer for fresh 4-cell-stage embryo transfers and on the designated day after the blastocyst transfer carried out during 2004–2005.

Days after day 2 embryo transfer *Days after blastocyst transfer*	*Day 0** *Day 0***	*Day 3*	*Day 4*	*Day5*	*Day 6*	*Day 7*	*Day 8*	*Day 12*
No. of cycles	3724 5048	1028	3679	960	1394	3671	1668	3327
% of cycles progesterone ≤10 ng/ml	27.7 13.1	21.8	17.1	30.8	7.7	41.5	24.5	74.4
% of cycles progesterone >10 and ≤20 ng/ml	43.0 74.7	63.6	54.9	46.5	21.4	20.5	28.7	3.8
% of cycles progesterone >20 ng/ml	29.2 12.2	14.6	28.0	22.7	70.9	38.0	46.9	21.8
Mean progesterone where β-HCG is ≤1.0 IU/l	16.6 14.8	14.3[a]	14.7[c]	10.2[d]	26.6[b]	6.2[e]	18.1[f]	2.4[g]
Mean progesterone where β-HCG is >1.0 IU/l	– –	13.9[a]	20.1[c]	18.7[d]	26.4[b]	25.0[e]	45.2[f]	40.6[g]
β-HCG (IU/l) where implantation occurred		0.5	3.2	9.4	0.6	40.0	10.4	99.5

The upper row of figures expresses the data regarding day 2 embryo transfer and the lower row that regarding blastocyst transfer.
Day 0* means 2 days post-ovulation; HCG = human chorionic gonadotrophin.
Day 0** means 4.8 days post-ovulation (blastocyst transfer was carried out on average on day 4.8).
[a,b]In statistical tests with respect to progesterone concentration, values with the same superscript letter were not significantly differerent (*t*-test).
[c,d,e,f,g]In statistical tests with respect to progesterone concentration, values with the same superscript letter were significantly different ($P < 0.0001$, *t*-test).

Discussion

Correlation between oestradiol concentration and number of oocytes

The number of matured follicles and the oestradiol concentration, and also the number of retrieved oocytes and the oestradiol concentration, were closely associated. Their ratio was 1.24–1.46, showing the result that the oestradiol concentration per retrieved oocytes was higher. This result demonstrated that the oestradiol concentration per matured oocytes needed to be approximately 300 pg/ml (**Table 3**). The date for administering the maturation trigger is naturally decided, based on the relationship between the number of matured follicles and the oestradiol concentration. The discrepancy of those two factors, however, became larger as age advanced and the number of retrieved oocytes expected from the oestradiol concentration became fewer (**Tables 3**, **4** and **6**).

LH surge

The emergency oocyte retrievals due to the premature onset of LH surge were performed according to the guideline shown in **Table 2**. Approximately 3.5% of all the oocyte retrieval cycles underwent emergency oocyte retrieval, being caught by the slight increase in the concentration of LH at the beginning of the LH surge. For such cases, GnRHa was immediately administered by a nasal spray, and oocyte retrieval was carried out after 28–30 h. Approximately 1.6% of all the oocyte retrieval cycles were cases where the oocytes were retrieved just before spontaneous ovulation after the LH surge had finished (**Table 5**). The ovulation rates were extremely low, 2.0 and 2.5% respectively. The oocyte retrieval rate was 60–70%, which was significantly lower than that for normal oocyte retrieval after the administration of GnRHa nasal spray ($P < 0.001$). This result suggests that the cases susceptible to premature LH surge have a high basal LH concentration (the basal LH concentration for emergency oocyte retrieval was 14.3 ± 7.0, compared with 7.3 ± 4.1 for normal oocyte retrieval, showing a significant difference; $P < 0.0001$), demonstrating a decrease in the level of basic ovarian functions which leads to a higher incidence rate of empty follicles.

Rate and number of oocytes retrieved

Both the rate and the number of oocytes retrieved decreased as the age advanced and opportunity to use HMG decreased in women of advanced age. The result threw doubt on the effectiveness of stimulation for women of advanced age.

It became clear that the administration of oral contraceptives in the previous cycle was effective for increasing the number of oocytes retrieved and embryos cleaved. In particular,

administration of oral contraceptives over the preceding two consecutive cycles showed a remarkable effect. Furthermore, it was found, from the relationship between the length of menstruation and the results of retrieved oocyte culture, that the group with a normal menstrual cycle of around 30 days had the highest number of oocytes retrieved and embryos cleaved and had a good grade embryo quality. This finding suggests the possibility that administration of oral contraceptives adjusts the menstrual cycle to a 30 day pattern while suppressing FSH and LH, giving a positive influence on the process of follicular maturation from the preantral follicle to the antral follicle.

Outcome of embryo transfer

The statistics, including the live birth rate, miscarriage rate and ectopic pregnancy rate, analysed here are all in relation to the total number of initiated cycles. According to age brackets, the number of embryos transferred tended to be higher in the younger age groups. This was because double embryo transfers were carried out until 2003. Since then, the mode has shifted to elective single embryo transfer and the number of embryos transferred was drastically reduced. The live birth rate for the fresh 4-cell-stage embryo transfer was 5.2%, and the combined rates for miscarriages and ectopic pregnancies was 2.0%. The live birth rate for frozen–thawed embryo transfer was 5.9%, and the combined rate for miscarriages and ectopic pregnancies was 1.7%. The final cumulative pregnancy rate for both embryo transfers was 14.8%. The live birth rate by age group for 39 and above decreased drastically to less than 10%, coupled with a decrease in the transfer rate in relation to oocyte retrieval cycles.

In the clomiphene citrate cycles where the follicular maturation is triggered by GnRHa, the β-HCG concentration can be measured accurately because exogenous HCG is not present in the body. **Figures 3a** and **3b** show the precise evaluation of the correlation between the live birth rate and the β-HCG concentration. The probability of subsequent events, including live birth, miscarriage and ectopic pregnancy, can be predicted with a considerable degree of accuracy with the day 12 β-HCG concentration for 4-cell-stage embryo transfer and the day 7 β-HCG concentration for blastocyst transfer. **Figure 3c** shows the rate of multiple pregnancies. The twin pregnancy rate was 11.52% and the triplet pregnancy rate was as low as 0.04%. In the clomiphene cycles where fewer oocytes were retrieved and thus fewer embryos were transferred, the occurrence of multiple pregnancies was naturally reduced.

Figure 4a shows the distribution of cycles in each endometrial thickness bracket, and **Figure 4b** shows the relationship between the endometrial thickness and the pregnancy rate. When restricting the age and the embryo grade under certain conditions, the distribution of the cycles shifts towards the thicker endometrial membrane group, and the pregnancy rate was further improved among the thinner group. Under the condition of 30–38 years old, top-grade embryo and single embryo transfer, 95% of the cycles belonged to the group with endometrial thickness of 8 mm or more, and the pregnancy rate reached 27% or more. This indicates that, besides clomiphene citrate, other factors such as the age of the patient and the number and quality of oocytes are involved in creating the thickness of the endometrial membrane. Therefore, the comprehensive functioning level of the uterus and the ovary in regard to pregnancy, is thought to be reflected in the thickness of the endometrial membrane. In fact, the distribution of the thickness of endometrial membrane at the time of transfer in the absolute natural cycle is almost identical to that of the clomiphene citrate cycle (**Figure 4a**). Although there is a suggestion that the pharmacological action of clomiphene citrate may be influencing the endometrial membrane at the time of implantation, and decreasing the membrane's embryo receiving ability, if this is the case, it should decrease the pregnancy rate regardless of the thickness of the endometrial membrane. However, the facts do not endorse this suggestion. Furthermore, although one of the isomeric components of clomiphene citrate, enclomiphene, acts antagonistically to the oestrogen receptor at the level of the hypothalamus, its action is not persistent, as is obvious from its short half-life of 24 h. Thus, it is logically impossible for it to be influencing the endometrial membrane at the time of implant, which is more than 7 days later than the final administration of clomiphene citrate. Based on these observations, the often-made suggestion that the effect of clomiphene citrate on the endometrial membrane decreases the pregnancy rate is contradicted by these impartially validated facts.

LH surge inhibiting action and endogenous LH maintenance action of clomiphene citrate

In the range where the oestradiol concentration was 2000 pg/ml or lower, the incidence of the LH surge was 5% or less. The reason for this may be that enclomiphene's antagonistic action on the oestrogen receptor strongly inhibits the positive feedback at the level of the hypothalamus (**Figure 5**) (Goldstein *et al.* 2000). However, enclomiphene's half-life is as short as 24 h or less, and as its action is thought to wane drastically within a few days after discontinuation of its administration, the conventional regimen where it is discontinued on day 9 will lead to the situation where an LH surge is easily initiated due to positive feedback from around day 13–14 (Mikkelson *et al.* 1986). The protocol addresses this situation and uses clomiphene citrate as a valid measure to inhibit the LH surge by continually administering clomiphene citrate until immediately before the follicular maturation is triggered. In addition, the spontaneous adjustment of endogenous LH in the clomiphene citrate cycle contributes to favourable embryonic development and normal steroid metabolism. Furthermore, triggering of the endogenous LH surge by GnRHa plays an important role in sustaining normal LH dynamics and normal luteal function in the luteal phase of the clomiphene citrate cycle.

Aim of continuous, prolonged administration of clomiphene citrate

At first, clomiphene citrate was developed as an oral contraceptive with the purpose of inhibiting ovulation. However, administering the drug for a few days or 5 days at the beginning of the period did not yield the expected result; on the contrary, however, light was thrown onto its ovulation induction action. Since then, clomiphene citrate has been used as the most fundamental drug for ovulation induction in the treatment of infertility up to the present. The treatment schedule for this drug, which has now been in use for 40 years, has not changed, and remains 5 days from day 5 of menstruation.

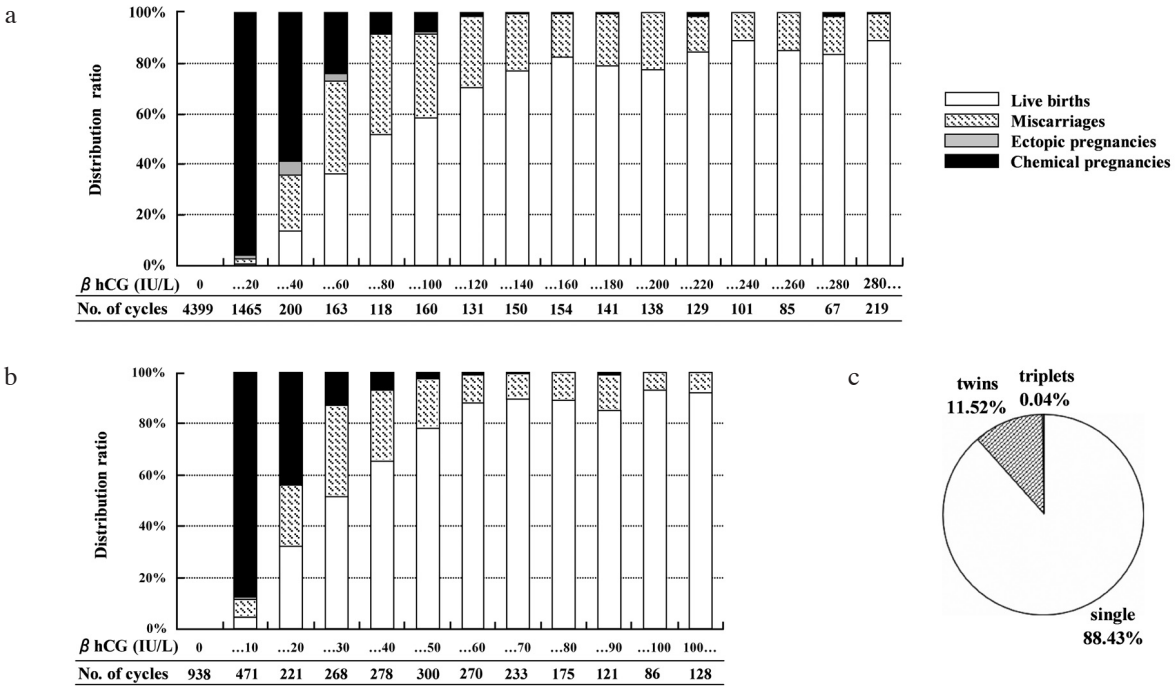

Figure 3. (**a**) Relationship between the concentration of β-HCG measured 12 days after embryo transfer and the rate of live births, miscarriages, ectopic pregnancies and chemical pregnancies for 4-cell-stage embryo transfer. (**b**) Relationship between the concentration of β-HCG measured 7 days after BT and the rate of live births, miscarriages, ectopic pregnancies and chemical pregnancies for blastocyst transfer. (**c**) Rate of multiple pregnancies.

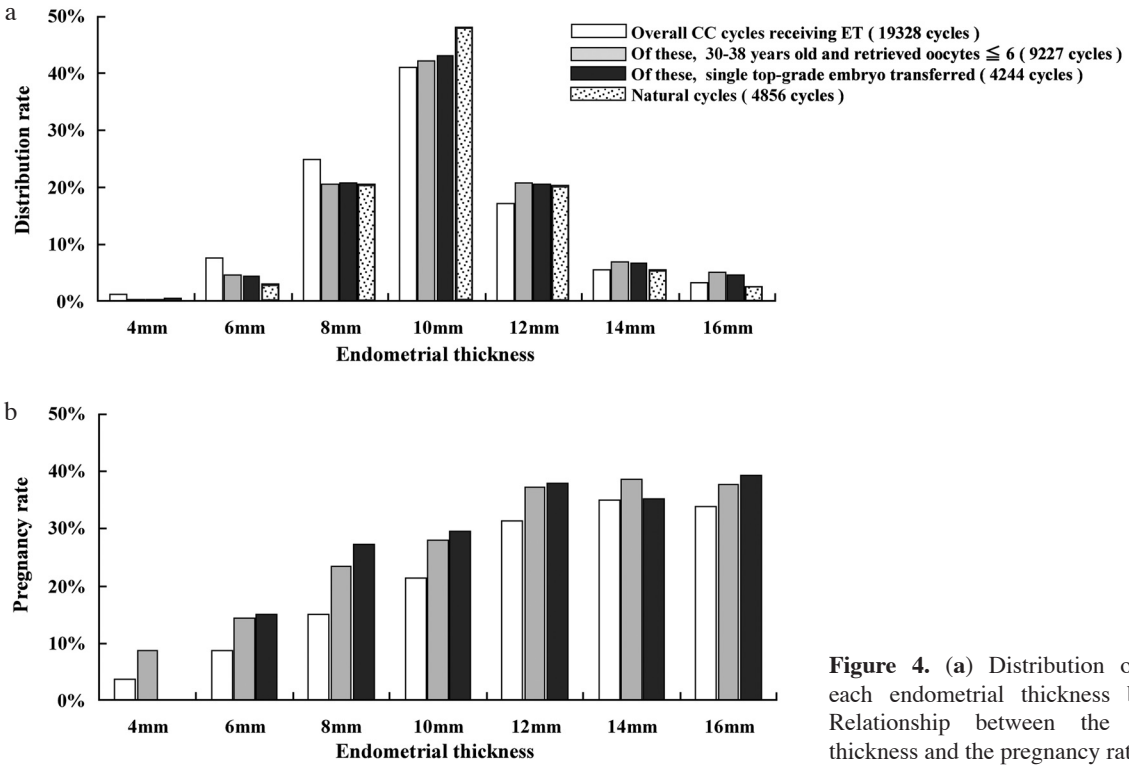

Figure 4. (**a**) Distribution of cycles in each endometrial thickness bracket. (**b**) Relationship between the endometrial thickness and the pregnancy rate.

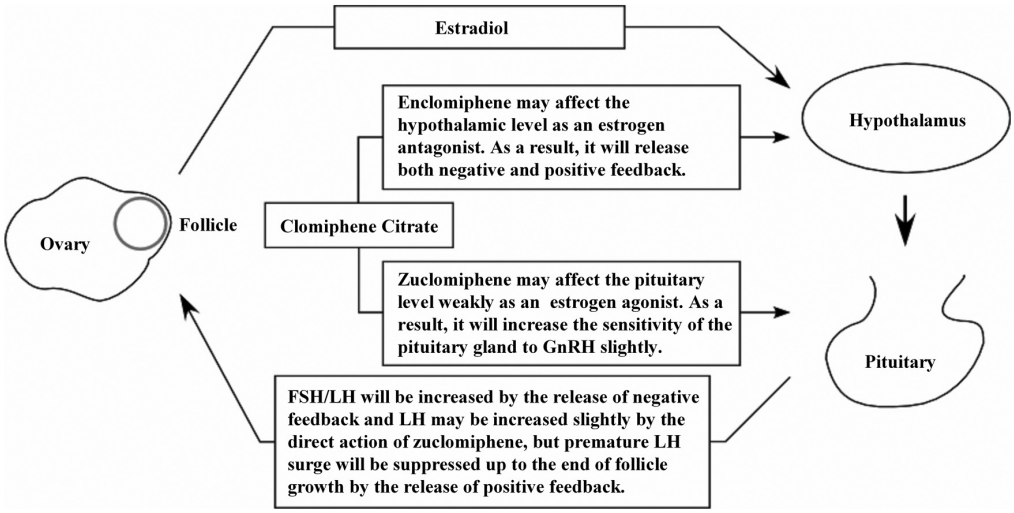

Figure 5. Hypothesis concerning the function of each isomer of clomiphene citrate.

Use of clomiphene citrate in IVF–embryo transfer was started in the clinic from 1995, and through this experience, it was observed that the incidence ratio of the LH surge decreased when clomiphene citrate was administered for more than 5 days, which was the usual regimen. This indicated that, for conquering the biggest problem in IVF–embryo transfer, that of premature LH surge, there may be a third method other than the conventional ovarian induction method using GnRHa or GnRH antagonist. This new method was thought to be revolutionary, in that it could prevent the premature LH surge while maintaining the pituitary functions, unlike the conventional method, which inhibits pituitary function.

Although the mechanism of action of clomiphene citrate is still shrouded in mystery, its anti-oestrogen action was expected to be the chief factor from early on. However, as early as 1973, an interesting report that went against this hypothesis was submitted, which highlighted the fact that the LH surge was not actually inhibited (Vanderberg *et al*., 1973). Since then, Adashi *et al*. have reported the outcome of their in-vitro study, stating that the target of action of clomiphene citrate was the pituitary and that it increases the oestrogen receptor at this site (Adashi *et al*., 1980); furthermore, the authors reported that enclomiphene binds itself to the receptor in competition with oestrogen at the pituitary level, and that its mode of action was oestrogenic (Adashi, 1984; Adashi *et al*., 1981). At this point, little attention was paid to the action of clomiphene citrate towards the hypothalamus, and it was thought that clomiphene citrate stimulated LH secretion by acting oestrogenically at the pituitary level.

However, in 1983, Okia confirmed that clomiphene citrate targets its action at the hypothalamus level, and that it increases the secretion of gonadotrophin by an unknown mechanism. He also referred to the fact that clomiphene citrate cancels the positive feedback and inhibits LH release at the pituitary level, although the mechanism was still unknown (Okia, 1983). In the same year, a report was published that referred to clomiphene citrate isomers and suggested that the enclomiphene acted on ovine pituitary cells as an oestrogen antagonist, contrary to the conclusions of Adashi *et al*. (Huang *et al*., 1983). Only then was the antagonistic action of clomiphene citrate isomers given attention.

The interesting thing about clomiphene isomers was their half-life. The fact that zuclomiphene has a far longer half-life compared with enclomiphene merits attention (Mikkelson *et al*., 1986). In 1988, Messinis *et al*. reported that prolonged administration of clomiphene citrate blocked positive feedback and prevented the LH surge (Messinis *et al*., 1988). However, the authors did not discuss the mechanism of these very interesting effects to the extent of further considering the correlation of the half life and the anti-oestrogenic function of the two clomiphene isomers.

In 1999, Young asserted that the accumulation action of zuclomiphene due to its long half-life was the driving force in the clomiphene's ovulation inducing action, and thus that the clomiphene citrate's action was mainly caused by zuclomiphene. Contrarily, in 2001 and 2006, Pakraski *et al*. reported that the main player for ovulation induction was enclomiphene, and that zuclomiphene was incapable of such action. The authors also highlighted the fact that enclomiphene's anti-oestrogenic action inhibits ovulation, and pointed out the necessity of administering HCG for ovulation induction.

As described above, there have been many reports and discussions concerning the actions of clomiphene citrate. However, it can be said that the discussions veered between the oestrogenic action and anti-oestrogenic action of clomiphene citrate. **Table 5** shows that the emergency oocyte retrieval and the post-LH surge oocyte retrieval consisted of only 5.1% of all oocyte retrievals, demonstrating that the LH surge did not take place in 94.9% of all oocyte retrievals. Furthermore,

the relationship between oestradiol concentration and LH concentration for this 94.9% on the day of maturation triggering is shown in **Table 10**. From this, it can be seen that the LH surge was effectively inhibited regardless of the elevation of the oestradiol concentration. These outcomes demonstrate that the anti-oestrogenic action of enclomiphene is the key player in the action of clomiphene citrate, and that this isomer with a short half-life can only be effective in cancelling the positive feedback at the hypothalamus level when used continuously for a long period. In other words, only when clomiphene citrate is administered continuously until the day before the maturation triggering can the onset of premature LH surge be inhibited extremely effectively.

This fact means that not only can it increase endogenous FSH/LH by facilitating the hypothalamus–pituitary axis, but also that it can stave off ovulation by inhibiting the premature LH surge. Therefore, this protocol can be said to be an ovulation induction method that integrates the contradictory actions of clomiphene citrate effectively.

Issues with regard to prolonged use of clomiphene citrate

Although there was a report describing the correlation between the prolonged, excessive use of clomiphene citrate and the occurrence of malignant ovarian tumours (Rossing *et al.*, 1994), the authors later denied the causal correlation (Rossing *et al.*, 2000) and, at present, the majority of studies suggest that there is no apparent correlation. However, a dose of more than 750 mg per cycle is thought not to be permissible, and close attention must be paid to the possibility of the occurrence of ovarian tumours. The mean dose of clomiphene citrate in this study was 575 mg and the mean number of cycles treated was 3.0 cycles. Fourteen per cent of the patients out of the 11,739 who were included in the present study had had IVF treatment with clomiphene citrate in the clinic between 1998 and 2000, prior to the present study. The mean number of treatments per patient was 3.67. Although the mean dose per cycle exceeded the mean dose per cycle in Japan (500 mg) by 75 mg, the regimen did not deviate from Japanese guidelines for the administration of clomiphene citrate. The study on the live births resulting from the fresh 4-cell-stage embryo transfers based on the clomiphene cycle revealed a tendency for the incidence of low birth weight infants and extreme immaturity to be more frequent than the Japanese average (data not shown). However, the remaining indices were not different from those for natural pregnancy. There is a necessity to carry out a follow-up investigation concerning the incident rates of uterine, ovarian and mammary cancers. Although data appropriate for statistical analysis have not yet been gathered, this is an important issue that needs to be addressed in the future.

Regimen for HMG or FSH administration

The aim of administering HMG/FSH was to reduce the number and dosage of the administration as far as possible. Therefore, HMG/FSH was administered every other day from day 8. The background of this regimen was the restriction on injecting drugs in Japan, where the injection of drugs is limited to within medical institutions and patients are not permitted to do so by themselves except for a limited number of drugs. Therefore, a reduction in the number of administrations means a reduction in the number of times that patients have to come to the clinic, something that benefits patients enormously. In addition, one of the advantages of natural cycles is that, by leaving the process of follicular development to a natural hormone environment, there is a greater likelihood of best quality oocytes developing into the dominant follicle. Although, this selection can be equally expected with the clomiphene cycles, the addition of HMG/FSH is thought to contribute more effectively to the development of several follicles, one of which can become the dominant follicle. In fact, there are many reports suggesting that the combined use of HMG/FSH can contribute to an improvement of the pregnancy rate with the clomiphene cycles (Dorn and Ven, 2005).

This protocol has been devised with the aim of achieving the optimal development of follicles with the least administration of HMG/FSH. However, the timing and dose largely depend on past experience. Therefore, the relevance of this protocol needs to be evaluated objectively by a future prospective study concerning the timing, dose and dosing interval of the administration.

Reasons for using GnRHa

The protocol applied to the cases described in the present study, which has been used since 2001, is based on the single nasal administration of 240 μg of GnRHa (buserelin acetate) as the maturation trigger, rather than an intramuscular injection of HCG at 5000–10,000 IU. There are five reasons for this: (i) to avoid ovarian hyperstimulation syndrome (OHSS) caused by HCG; (ii) to maintain natural luteal function; (iii) to have a natural maturing process of oocytes based on the endogenous LH/FSH surge; (iv) to avoid incomplete follicular atresia caused by the HCG apotosis inhibiting activity on granulose cells; and (v) to avoid the adverse effect on folliculogenesis due to a luteal function which is overstimulated by HCG.

There was no severe case of OHSS that required hospitalization. There were also hardly any cases of moderate OHSS, which made it impossible for patients to go to work following the administration. Therefore, avoidance of OHSS by this method can be said to be extremely effective.

There have been many reports on the oocyte quality and the luteal function in IVF where GnRHa was used as the final maturation trigger, comparing the results with cases with HCG (Gerris *et al.*, 1995; Humaidan *et al.*, 2005; Griesinger *et al.*, 2006). Many of these reports conclude that cycles where GnRHa was used as the maturation trigger bear comparison to HCG cycles in the number of metaphase II (MII) oocytes obtained. However, they also conclude that the pregnancy rate is lowered and the rate of early miscarriages increases in the GnRHa cycles. This has been attributed to the early lowering of progesterone due to luteal phase insufficiency. However, these evaluations were made for the cotreatment cycles where a large dose of FSH and GnRH antagonist were used, and they are fundamentally irrelevant to the present study. In other words, under the environment where factors qualifying the luteal function other than GnRHa exist, it cannot be verified whether GnRHa *per se* is the cause of luteal phase insufficiency. In fact, in a similar study using only clomiphene citrate for ovarian induction and using GnRHa as the maturation trigger, no significant difference

was found with HCG cycles in the maintenance of progesterone concentration until the mid-luteal phase (Schmidt-Sarosi *et al*., 1995). Furthermore, in a study using GnRHa as the maturation trigger after cotreatment with clomiphene citrate and HMG, the pregnancy rate was better than when using HCG (Emperaire *et al*., 1992).

In this study, the progesterone concentration was measured in principle for cases of fresh 4-cell-stage embryo transfer carried out during 2004–2005, at the time of embryo transfer, on days 6 or 8, and on day 12. As a result, as shown in **Table 11**, the progesterone concentration rose from the time of embryo transfer to day 6, stayed at the highest concentration from days 6 to 8, and declined when there was no rise in the HCG concentration due to implantation failure, and was reduced to 5 ng/ml or less in 86% of cases on day 12. The rise in the concentration of HCG due to implantation began during days 6 and 8 of embryo transfer. Comparison of the progesterone concentration between two groups divided on the basis of whether day 8 HCG concentration was 1.0 IU/l and above, or less, showed a significant difference of 18.1 versus 45.2 ng/ml ($P < 0.001$). These facts reflected distinctly the natural fate of luteal function where normal implantation begins by day 8 of embryo transfer, the elevated HCG stimulates corpus luteum to form corpus luteum verum, the progesterone concentration rises but starts declining after day 8, when implantation does not take place, and disappears on around day 12 of embryo transfer or day 14 of ovulation.

Frozen–thawed blastocysts were transferred on day 4 or 5 (4.8 ± 0.5 days) of natural ovulation induced by a single nasal administration of GnRHa and the progesterone concentration was measured on the day of embryo transfer, on days 3 and 5 or on day 4, and on day 7. As shown in **Table 11**, the progesterone concentration remained at its highest from the time of embryo transfer to day 4, declined afterwards when HCG concentration was not raised due to implantation failure, and became 5 ng/ml or less in 49% of cases on day 7 of embryo transfer. The rate of cases where the progesterone concentration was 5 ng/ml or less was low at the beginning, namely 0, 3.9 and 4.1% on the day of embryo transfer, day 3 and day 4 respectively. However, the rate increased to 11.0 and 30.4% on days 5 and 7 respectively. The rise in the concentration of HCG due to normal implantation began from day 4 of embryo transfer. Comparison of the progesterone concentration between two groups divided on the basis of whether day 5 HCG concentration was 1.0 IU/l and above, or less, showed a significant difference of 10.2 versus 18.7 ng/ml ($P < 0.001$). These facts reflected distinctly the natural fate of the luteal function where normal implantation begins by around day 4 of embryo transfer, the elevated HCG stimulates corpus luteum to form corpus luteum verum, the progesterone concentration rises but starts declining after day 5 when implantation does not take place, and is reduced to less than half of its highest concentration on day 7 of embryo transfer or day 12 of ovulation.

From the above, it has become clear that the progesterone concentration after ovulation induction by GnRHa followed a distinctly natural course, regardless of whether it was a clomiphene citrate cycle or natural ovulation cycle, and that it maintained the highest concentration until the time normal implantation commenced and rose even higher in the case of successful implantation and otherwise declined. Therefore, it can be concluded that ovulation induction by GnRHa can induce a normal luteal function regardless of whether it is used alone or in combination with clomiphene citrate.

Perhaps the luteal function insufficiency following ovulation induction by GnRHa reported so far was a result that had been influenced by the inhibition of endogenous LH caused by the prior prolonged use of GnRH antagonist and not caused by the GnRHa *per se*.

The significance of the FSH surge in relation to the follicular maturation has long been pointed out (Fauser *et al*., 2002). However, a concrete conclusion has not been reached yet. Although the present study cannot discuss this point, as it is not intended as a comparative study, from the point of view of the rate of mature follicles (MII follicles) obtained, as shown in **Table 6**, GnRHa seems to be as effective a maturation trigger as HCG.

In order to discuss concretely the advantages of GnRHa over HCG, further studies are necessary, involving discussion of the process of follicular atresia and GnRHa influence on folliculogenesis (iv and v). In this regard, the likelihood of HCG preventing the apoptosis of granulose cells (Park *et al*., 2003) and allowing follicles that have failed to undergo atresia to persist is worthy of attention.

It has become clear that use of GnRHa as the final maturation trigger raises no problem in obtaining matured follicles and maintaining the subsequent luteal function. Furthermore, it is expected that its level of interference with the process of follicular atresia and of having an adverse effect on folliculogenesis are lower than those for HCG. Thus, it can be concluded that further studies with regard to its clinical application will prove of great importance.

Conclusions

The efficacy of ovarian stimulation using clomiphene citrate in conjunction with HMG/FSH has been evaluated in many studies (Dorn *et al*., 2005). They are based on a common regimen where clomiphene citrate is administered from day 5 to day 9 at 50–150 mg/day, and GnRH antagonist is used in the latter half of the follicular phase in order to prevent a premature LH surge. Although the role of clomiphene citrate in this method is to work as an anti-oestrogen in order to increase endogenous FSH and thus to increase the number of developed follicles, in reality, this purpose seems to be lost due to the large dose of HMG/FSH used in conjunction. The protocol used in this study differs from these protocols in that clomiphene citrate was used for a purpose other than ovarian stimulation. It was used for inhibiting the premature LH surge while maintaining pituitary function. In addition, because the cycles were not down-regulated, induction of the endogenous LH surge by GnRHa was possible, and therefore the preventive effect for OHSS could also be expected. Furthermore, it is also noteworthy that this could avoid the administration of non-physiological HCG, which does not exist *in vivo* in women when not pregnant. In other words, this protocol can be expected to reduce the disruption of in-vivo folliculogenesis and steroidogenesis by avoiding the administration of HCG.

In this study, the possible efficacy of hormone adjustment for two cycles by oral contraceptives prior to the initiation of the clomiphene cycle has been suggested in order to increase the rate of oocyte retrieval. However, this interesting result needs to be confirmed by future prospective studies.

The advantage of prolonged, consecutive use of clomiphene citrate lies in the reduction of the dose of HMG/FSH. The result undoubtedly leads to an alleviation of the financial and physical burdens on patients. For example, in the present analysis, in the age bracket 38 years old or younger, 53% of patients had live babies after the mean trial number of 2.5 cycles and the mean cost was £3200. Perhaps, in many advanced countries, one needs to spend more to obtain a live baby through IVF–embryo transfer at a probability of 50%. It may also be impossible for women in less-developed countries to manage to pay such high fees while working and having treatment. In view of this protocol being inexpensive and making work and treatment compatible, it can be expected to provide a large benefit to infertile women in developing countries, in particular.

Furthermore, another benefit of this low stimulation method is that it interferes less with the in-vivo hormone conditions. This difficult-to-confirm result can be beneficial to women whose ovarian functions are caused to wither in the course of IVF treatment. In the future, through strict investigation into the correlations between the number of treatments and the number and quality of obtained oocytes, this protocol also needs to be evaluated from the point of view of protecting ovaries.

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Paper based on a contribution presented at the First World Congress on 'Natural Cycle/Minimal Stimulation IVF' in London, UK, December 15–16, 2006.

Received 14 December 2006; refereed 23 January 2007; accepted 11 May 2007.