Prognostic factors associated with clinical pregnancy in *in vitro* fertilization using pituitary down-regulation with depot and daily low-dose luteal phase gonadotropin releasing hormone agonists: A single center's experience

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ABSTRACT

AIM: To review the experience on depot-dose, and daily low-dose gonadotropin releasing hormone agonist (GnRHa) long protocols and identify prognostic factors. **SETTING AND DESIGN:** A chart review was conducted on 2106 depot and 1299 daily low-dose cycles at a university hospital. **METHODS:** Clinical parameters were summarized, and prognostic factors of clinical pregnancy for each protocol were identified by logistic regressions. Missing data were imputed using multiple imputations (MI) and the regression models were rerun after MI. **RESULTS:** Clinical pregnancy rate was 57.5% and 46.9% in the depot and daily low-dose groups, respectively. Logistic regressions with MI identified age (odds ratio [OR]: 0.95, 95% confidence interval [CI]: 0.92–0.98), serum progesterone (OR: 0.62, 95% CI: 0.45–0.84) and endometrial thickness (OR: 1.06, 95% CI: 1.02–1.12) on human chorionic gonadotropin (hCG) day, number of oocytes retrieved (OR: 1.04, 95% CI: 1.01–1.06), fertilization rate (OR: 2.66, 95% CI: 1.46–4.87) and ratio of good-quality D3 embryos (OR: 4.31, 95% CI: 2.79–6.67) as prognostic factors in the depot group. Age (OR: 0.95, 95% CI: 0.92–0.98), endometrial thickness on hCG day (OR: 1.09, 95% CI: 1.03–1.15), ratio of good quality D3 embryos (OR: 2.56, 95% CI: 1.59–4.13) and the number of cryopreserved embryos (OR: 1.07, 95% CI: 1.003–1.15) are prognostic for the daily low-dose protocol. Some regression coefficients that are significant under model-wise deletion become nonsignificant after MI. **CONCLUSIONS:** Age, embryo quality and endometrial thickness on hCG day are important prognostic factors for both 1.0/1.3 mg depot and 0.05/0.1 mg daily low-dose luteal phase GnRHa long protocols. MI is a valuable tool to gauge and address bias caused by missing data in reproductive medicine.

KEY WORDS: Controlled ovarian stimulation, gonadotropin releasing hormone agonist, missing data, multiple imputations, pituitary down-regulation

INTRODUCTION

For pituitary down-regulation, depot gonadotropin releasing hormone agonist (GnRHa) protocol is more convenient to use while daily low-dose protocol allows more rapid recovery of pituitary response after withdrawal and confers greater flexibility.[1] Dosages of GnRHa range from 1.88 (half-dose) to 3.75 mg (full dose) in the depot protocol and from 0.05 to 0.5 mg in the daily low-dose protocol.[2] Further, reduction of depot GnRHa to one-third-dose (1.25 mg) can mitigate excessive pituitary suppression and may potentially reduce treatment cost.[3] In light of this, it will be interesting to compare the one-third-dose depot and the daily low-dose protocols. However, multicenter randomized controlled trial (RCT) data are not yet available. We performed a retrospective analysis for the 1.0/1.3 mg depot-dose and the 0.05/0.1 mg daily low-dose GnRHa protocols and explored prognostic factors for each, aiming to inform clinical practice using a large dataset.

METHODS

We reviewed the *in vitro* fertilization (IVF)/intracytoplasmic sperm injection (ICSI) cycles administered between June 2010 and October 2013 where luteal phase GnRHa down regulation was used. Ethics approval was obtained from the Institutional Review Board and all the patients gave written informed consents for data extraction for research. Cases were excluded from analysis if any of the following criteria applied: (1) Female partner was 45 years or older; (2) gamete donation or *in vitro* maturation; (3) premature ovarian failure, recurrent implantation failure (failure to conceive after three or more IVF attempts or transfer of ten or more good quality embryos) or recurrent IVF failure;[4] (4) congenital or acquired uterine anomalies, regardless of whether it was surgically repaired; (5) co-transfer of the thawed embryos from previous cycles; (6) estradiol valerate or hormone replacement therapy priming prior to gonadotropin (Gn) stimulation; (7) growth hormone supplementation; (8) co-transfer of cleavage-stage embryos and blastocysts. To meet the independent assumption for logistic regression, only the first eligible controlled ovarian stimulation (COS) cycle for each included couple was analyzed. Some of the included couples had undergone IVF/ICSI elsewhere before being referred to this clinic.

Standard mid-luteal phase GnRHa long protocols were administered. In the depot dose protocol, triptorelin (Dipherelin, Beaufour Ipsen or Decapeptyl, Ferring) was given as 1.0 or 1.3 mg intramuscularly once per cycle. In the daily low-dose protocol, triptorelin was given as 0.1 or 0.05 mg subcutaneously once per day until the day of ovulation trigger. In some cycles, daily triptorelin injections were commenced at 0.1 mg level and were then tapered to 0.05 mg/day from the 1st day of the ensuing period. Recombinant follicle-stimulating hormone (Gonal-F, Serono or Puregon, MSD), highly-purified human menopausal gonadotropin (Menopur, Ferring or HMG, Livzon) or urofollitropin (Fostimon, IBSA or Purified Urofollitropin, Livzon) were then prescribed based on the patient's history and the physician's judgment. After 5 days of stimulation, doses were adjusted based on follicular growth and recombinant luteinizing hormone (LH) (Luveris, Serono) was supplemented as indicated by staggered follicular growth and suppressed LH (<0.5 IU/L). Ovulation was induced with human chorionic Gn (Ovidrel 250 μg, Serono or hCG 10,000 IU, Livzon) when at least two follicle were ≥18 mm in diameter. Oocytes were retrieved 36–37 h later by ultrasound-guided transvaginal aspiration.

Retrieved oocytes were inseminated by IVF, ICSI, or 50% IVF + 50% ICSI in rare cases. Embryos that were comprised of ≥6 equally sized blastomeres and had 20% of fragmentation at 68 h after fertilization were classified as good quality cleavage-stage embryos eligible for transfer or cryopreservation.[5] Blastocysts were evaluated on the 5th and 6th days according to developmental status of the inner cell mass and trophectoderm following the Gardner *et al*. grading scale.[6] Blastocysts with blastocoeles that extended to at least half of the volume of the embryos were eligible for fresh transfers, while those with blastocoeles that completely filled the entire embryos were eligible for cryopreservation.

Cleavage-stage embryo or blastocyst transfer was chosen based on the patients' history and physicians' judgment. Two or three embryos were transferred on day 3 after ovum pick-up (OPU) for cleavage-stage embryo transfer, while no >2 embryos were transferred on day 5 after OPU when blastocysts were transferred. Embryo transfers were cancelled for patients who were at significant risk for ovarian hyperstimulation syndrome, patients with exceedingly thin endometrium (<0.6 cm) or premature rise of progesterone ($P \geq 1.5$ ng/ml) on the human chorionic gonadotropin (hCG) day.

The primary outcome of this study is clinical pregnancy rate, which is the proportion of embryo transfer cycles that resulted in ultrasonographic visualization of at least one gestational sac, regardless of the location. Multiple endocrine and embryological parameters were also measured.

We summarized the normally distributed continuous variables with mean and standard deviation; continuous variables with skewed distributions were summarized with median and interquartile range. Categorical variables were described with numbers and percentages. Univariable comparisons were made with student's t-test or Wilcoxon rank-sum test for continuous variables and Chi-squared test or Fisher's exact test for binary variables. Multivariable logistic regressions were performed to identify prognostic factors in each of the two treatment groups, which were restricted to the cycles with fresh embryo transfers. Continuous and binary predictor variables reported in previous studies were used for a preliminary model selection[7,8] [Table 1]. First, model-wise deletion (also known as complete case analysis) was used for model selection, during which cycles with missing data on any of the relevant variables was automatically dropped. Forward and backward stepwise model selections were conducted separately for each GnRHa group. Hosmer-Lemeshow goodness-of-fit test and C-statistics were used to examine model accuracy. A variance inflation factor (VIF) were calculated, where VIF >10 were used to as an indicator of collinearity.

Missing data were then imputed using multiple imputations (MI) with chained equations.[9,10] Covariates retained in the selected regression models were all imputed

using predictive mean matching for continuous variables and logistic functions for binary variables. The results of regressions with and without MI were compared. All the data analyses were performed with Stata Statistical Software (Release 13.1, by StataCorp LP, College Station, Texas, USA) and statistical significance was defined by two-sided $P < 0.05$.

RESULTS

3614 COS cycles using depot or daily low-dose GnRHa protocols for down-regulation were identified. After the exclusion criteria had been applied, 3405 cycles remained in the analysis, among which 2106 used depot protocol and 1299 used daily low-dose protocols. Clinical pregnancy was examined in 2271 cycles with embryo transfers.

Table 1: Candidate variables included the stepwise logistic regression model selection

Category	Variables
Continuous	Age (year); BMI (kg/m^2); duration of infertility (year); bilateral AFC; basal serum FSH (IU/L); duration of down-regulation[a] (day); serum LH concentrations on Gn initiation; duration of Gn stimulation (day); total Gn dosage (IU); serum LH (IU/L), *P* (ng/ml) and endometrium thickness (mm) measured on the hCG day; number of retrieved oocytes; number of transferred embryos; fertilization rate; ratio of good-quality embryos; number of cryopreserved embryos
Binary[b]	Primary infertility; secondary infertility; pelvic inflammatory disease; endometriosis/adenomyosis; PCOS/chronic anovulation; male factor infertility; unexplained infertility; utilization of rFSH, highly-purified urinary FSH, HMG, recombinant LH, OC priming; fertilization techniques (IVF, ICSI or rescue ICSI); blastocyst transfer

AFC=Antral follicular count, FSH=Follicle-stimulating hormone, LH=Luteinizing hormone, Gn=Gonadotropin; hCG=Human chorionic gonadotropin, P=Progesterone, PCOS: Polycystic ovary syndrome, rFSH=Recombinant follicular stimulating hormone, HMG=Human menopausal gonadotropin, OC=Oral contraceptive, IVF=*In vitro* fertilization, ICSI=Intracytoplasmic sperm injection. [a]The number of days from initiation of GnRHa to ovulation trigger, [b]All of the variables were coded as Yes-1, No-0

The depot group were observably younger, had shorter duration of infertility, higher proportion of primary infertility and higher basal antral follicular count, which was echoed by the difference in endocrine and embryological outcomes [Tables 2-4]. In total, 64.1% of the patients in the depot group underwent fresh embryo transfers, 57.5% of whom achieved clinical pregnancies. In contrast, among the daily low-dose group, 71.0% of the patients underwent fresh embryo transfers, and 46.9% of the transfer cycles resulted in clinical pregnancies. Follow-up was completed in 1687 cycles where embryo transfers were performed. Data from these cycles show that 45% and 37.7% of the embryo transfer cycles resulted in live births in the depot and daily low-dose groups, respectively.

The regression model selected by the forward method in the depot group included age, pelvic inflammatory disease, oral contraceptive (OC) priming, serum *P* and endometrial thickness on hCG day, number of transferred embryos and ratio of good quality embryos as observed on day 3. The model selected by the backward method included number of retrieved oocytes and fertilization rate apart from all the variables selected by the forward method [Table 5]. The model identified by the backward method was adopted as the two additional variables are biologically plausible. With model-wise deletion, 6% of the overall cases were dropped due to missing data in the depot group. Older age, OC priming and higher serum P on hCG day are associated

Table 2: Baseline demographic characteristics[a]

	Depot (*n*=2106)	Daily low-dose (*n*=1299)	Total (*n*=3405)
Age (years), mean (SD)‡	30.4 (3.8)	33.1 (4.2)	31.4 (4.2)
BMI (kg/m^2), median (IQR)	21.5 (19.9-23.6)	21.6 (20.1-23.5)	21.6 (20.0-23.6)
Primary infertility, *n* (%)‡	1076 (51.2)	544 (42.0)	1620 (47.7)
Infertility factor, *n* (%)			
Pelvic inflammatory disease	1477 (70.1)	925 (71.2)	2402 (70.5)
Male factor‡	607 (28.8)	303 (23.3)	910 (26.7)
Unexplained†	340 (16.1)	159 (12.2)	499 (14.7)
Endometriosis/adenomyosis†	232 (11.0)	191 (14.7)	423 (12.4)
PCOS or chronic anovulation disorders‡	291 (13.8)	98 (7.5)	389 (11.4)
Cervical factor	7 (0.3)	2 (0.2)	9 (0.3)
History of poor response*	0 (0.0)	4 (0.3)	4 (0.1)
Duration of infertility (year), median (IQR)‡	3 (2-5)	4 (2-7)	4 (2-6)
Ovarian reserve†, median (IQR)			
Basal FSH (IU/L)‡	5.9 (5.0-7.1)	6.2 (5.2-7.5)	6.0 (5.0-7.2)
Basal LH (IU/L)‡	3.9 (2.8-5.5)	3.5 (2.4-4.8)	3.7 (2.6-5.2)
Basal E_2 (pg/ml)	38.0 (28.0-49.6)	38.3 (28.0-52.7)	38.0 (28.0-50.4)
Bilateral AFC‡	15 (12-20)	11 (8-14)	13 (10-18)

[a]Continuous variables were described using mean and SD if normally distributed or with median and IQR if otherwise. SD=Standard deviation, IQR=Interquartile range, PCOS=Polycystic ovary syndrome, FSH=Follicle-stimulating hormone, LH=Luteinizing hormone, E_2=Estradiol, AFC=Antral follicular count, *P<0.05 (by Fisher's exact test), †P ≤ 0.01, ‡P ≤ 0.001.

Table 3: Controlled ovarian stimulation treatment attributes[a]

	Depot (*n*=2106)	Daily low-dose (*n*=1299)	Total (*n*=3405)
OC priming, *n* (%)‡	124 (5.9)	44 (3.4)	168 (4.9)
Down-regulation duration (days), median (IQR)[b]‡	26 (25-28)	25 (24-26)	26 (25-28)
LH after down-regulation (IU/L), median (IQR)‡	1.3 (0.9-1.9)	1.2 (0.8-1.8)	1.3 (0.9-1.9)
Gn duration (days), median (IQR)‡	12 (11-13)	11 (10-12)	11 (10-12)
Total Gn dose (IU), median (IQR)‡	2000 (1650-2475)	2250 (1800-2700)	2100 (1650-2600)
hCG day parameters[c]			
LH (IU/L), median (IQR)‡	0.8 (0.5-1.2)	1.1 (0.7-1.7)	0.9 (0.6-1.4)
E_2 (pg/ml), median (IQR)‡	3305 (2307-4300)	2938 (1952-4288)	3162 (2164-4300)
P (ng/ml), median (IQR)*	0.8 (0.6-1.1)	0.8 (0.5-1.1)	0.8 (0.6-1.1)
P>1.5 ng/ml, *n* (%)	601 (29.8)	327 (26.4)	928 (28.5)
Endometrium thickness (mm), median (IQR)‡	12 (10-14)	11 (10-13)	12 (10-13)
OPU cancellation, *n* (%)†	17 (0.8)	25 (1.9)	42 (1.2)
Moderate/severe OHSS, *n* (%)‡	137 (6.5)	43 (3.3)	180 (5.3)

[a]Continuous variables were described using mean and SD if normally distributed or with median and IQR if otherwise; [b]Number of days from the 1st day of GnRHa down-regulation to ovulation trigger with hCG, excluding those whose ovum retrievals were cancelled. OC=Oral contraceptive, IQR=Interquartile range, SD=Standard deviation, GnRHa=Gonadotropin releasing hormone agonist, Gn=Gonadotropin, LH=Luteinizing hormone, hCG=Human chorionic gonadotropin, E_2=Estradiol, P=Progesterone, OPU=Ovum pick-up, OHSS=Ovarian hyperstimulation syndrome, *P<0.05, †P≤0.01, ‡P≤0.001

Table 4: Embryology and pregnancy outcomes[a]

	Depot (*n*=2106)	Daily low-dose (*n*=1299)	Total (*n*=3405)
Number of oocytes retrieved, median (IQR)[b]	14 (10-19)	10 (7-14)	13 (9-17)
Number of oocytes inseminated, median (IQR)[c]	14 (9-18)	10 (6-13)	12 (8-16)
Methods of insemination, *n* (%)[c]			
IVF	1488 (71.3)	948 (74.7)	2436 (72.6)
ICSI	586 (28.1)	317 (25.0)	903 (26.9)
Rescue ICSI	12 (0.6)	5 (0.4)	17 (0.5)
Fertilization rate (%)[1], median (IQR)[c]	70.6 (56.5-82.1)	70.0 (50.0-83.3)	70.5 (54.5-83.3)
Good quality embryo rate (%)[2], median (IQR)[c]	66.7 (45.5-83.3)	60.0 (33.3-80.0)	64.3 (41.7-83.3)
Patients with fresh embryos transferred, *n* (%)	1349 (64.1)	922 (71.0)	2271 (66.7)
Number of embryos transferred, mean (SD)[d]	2.0 (0.4)	2.1 (0.5)	2.1 (0.5)
Biochemical pregnancy[3], *n* (%)[d]	830 (61.5)	467 (50.7)	1297 (57.1)
Clinical pregnancy[4], *n* (%)[d]	775 (57.5)	432 (46.9)	1207 (53.2)
Early miscarriage[5], *n* (%)[e]	54 (7.0)	37 (8.6)	91 (7.5)
Ectopic pregnancy[6], *n* (%)[e]	18 (2.3)	15 (3.5)	33 (2.7)
Multiple pregnancy[7], *n* (%)[e]	314 (40.5)	151 (35.0)	465 (38.5)
Live birth[8], *n* (%)[f]	443 (45.0)	265 (37.7)	708 (42.0)

[a]The continuous variables were described using median and IQR due to nonnormal distribution, [b]For the 2089 depot and 1274 daily low-dose cycles with ovum pick-up, [c]For the 2086 depot and 1270 daily low-dose cycles with oocyte inseminations, [d]For the 1349 depot and 922 daily low-dose cycles with embryo transfers, [e]For the 775 depot and 432 daily low-dose cycles with confirmed clinical pregnancies, [f]For the 1687 embryo transfer cycles (depot=984, daily low-dose=703) with complete follow-up data of live birth. Definitions=1. 2 PN embryos/total inseminated oocytes per patient; 2. Good quality embryos as observed on day 3 after insemination/total 2 PN embryos per patient; 3. Biochemical pregnancies/transferred cycles; 4. Clinical pregnancies/transferred cycles; 5. Early miscarriage/confirmed clinical pregnancies; 6. Ectopic pregnancies/confirmed clinical pregnancies; 7. Twinning or higher order pregnancies/confirmed clinical pregnancies; 8. Live births/embryo transfer cycles, follow-up completed for 1687 embryo transfer cycles. IQR=Interquartile range, SD=Standard deviation, IQR=Interquartile range, IVF=*In vitro* fertilization, ICSI=Intracytoplasmic sperm injection, PN=Pronucleus

with a lower pregnancy rate, while increases in endometrial thickness on hCG day, number of oocytes retrieved, fertilization rate and ratio of good quality embryos are associated with a higher clinical pregnancy rate.

The model selected for the daily low-dose group included age, serum LH concentrations on the 1st day of Gn stimulation, serum P and endometrial thickness on hCG day, ratio of good-quality embryo observed on day 3, numbers of transferred and cryopreserved embryos, which is consistent in both forward and backward methods [Table 6]. With model-wise deletion, 20.8% of the cycles with embryo transfers in this group were dropped due to missing data. Older age and higher serum *P* on hCG day are associated with a lower pregnancy rate, while increases in serum LH on Gn initiation, endometrial thickness on hCG day, ratio of good quality embryos on day 3 and number of cryopreserved embryos are associated with a higher clinical pregnancy rate.

The most significant missingness was found in serum LH concentration on day 1 of Gn stimulation (16.9% and 15.6% missing in depot group versus daily low-dose group) and E_2 concentration on the hCG day (16.5% and

Table 5: Univariable and multivariable logistic regressions for clinical pregnancy outcome in the depot GnRHa group[a]

Covariate	Unadjusted OR[c]	95% CI	Adjusted OR[d]	95% CI	Adjusted OR[e]	95% CI
Age (years)	0.95‡	0.92, 0.97	0.96†	0.92, 0.99	0.95†	0.92, 0.98
Pelvic inflammatory disease[b]	0.83	0.66, 1.06	0.81	0.62, 1.05	0.84	0.65, 1.07
OC priming[b]	0.67	0.44, 1.04	0.60*	0.38, 0.94	0.64	0.41, 1.00
P on hCG day (ng/ml)	0.59‡	0.44, 0.78	0.57‡	0.41, 0.78	0.62†	0.45, 0.84
Endometrium thickness on hCG day (mm)	1.07†	1.02, 1.12	1.06*	1.01, 1.11	1.06†	1.02, 1.12
Number of oocytes retrieved	1.03*	1.00, 1.05	1.04‡	1.02, 1.07	1.04†	1.01, 1.06
Number of embryos transferred	1.18	0.89, 1.57	1.30	0.94, 1.80	1.24	0.90, 1.69
Fertilization rate[1]	2.84‡	1.62, 4.98	2.70†	1.45, 5.01	2.66‡	1.46, 4.87
Ratio of good quality embryos[2]	4.5‡	2.96, 6.85	4.29‡	2.73, 6.72	4.31‡	2.79, 6.67

[a]Restricted to the 1349 depot GnRHa cycles with fresh embryo transfers, [b]Coded as 1-Yes, 0-No, [c]Conducted with model-wise deletion, [d]With model-wise deletion, 1268 cycles were retained and 6% of the GnRHa depot cycles with embryo transfer were dropped due to incomplete information on any predictor variable. Chi-squared for Hosmer-Lemeshow Goodness-of-Fit test was 8.08 and *P* value was 0.57, C-statistic was 0.66. VIF ranged from 1.01 to 1.20 for all included covariates, [e]From the complete dataset, where missing data were imputed using multiple imputations with chained equations. All the 1349 cycles were included. VIF ranged from 1.01 to 1.20 for all the included covariates. Definitions=1. Proportion of normally fertilized oocytes (indicated by presence of two pronuclei 18 h after insemination) within all the oocytes that were inseminated; 2. Proportion of good quality embryos as observed on day 3 after insemination/total 2 PN embryos per patient. OC=Oral contraceptive, P=Progesterone, hCG=Human chorionic gonadotropin hormone, OR=Odds ratio, CI=Confidence interval, VIF=Variance inflation factor, GnRHa=Gonadotropin releasing hormone agonist. **P*<0.05, †*P*≤0.01, ‡*P*≤0.001

Table 6: Univariable and multivariable logistic regressions for clinical pregnancy outcome in the daily low-dose GnRHa group[a]

Covariate	Unadjusted OR[b]	95% CI	Adjusted OR[c]	95% CI	Adjusted OR[d]	95% CI
Age (y)	0.94‡	0.91, 0.97	0.93‡	0.90, 0.97	0.95‡	0.92, 0.98
Serum LH on Gn initiation (IU/L)	1.2	0.99, 1.44	1.23*	1.01, 1.49	1.19	0.99, 1.44
P on hCG day (ng/ml)	0.93	0.73, 1.18	0.60*	0.40, 0.90	0.92	0.71, 1.18
Endometrium thickness on hCG day (mm)	1.11‡	1.05, 1.17	1.11‡	1.04, 1.17	1.09†	1.03, 1.15
Number of embryos transferred	1.17	0.91, 1.49	1.37	1.00, 1.87	1.31	1.00, 1.71
Ratio of good quality embryos[1]	2.99‡	1.91, 4.68	2.14†	1.24, 3.69	2.56‡	1.59, 4.13
Number of cryopreserved embryos	1.13‡	1.06, 1.20	1.09*	1.01, 1.19	1.07*	1.003, 1.15

LH=Luteinizing hormone, Gn=Gonadotropin, P=Progesterone, hCG=Human chorionic gonadotropin hormone, OR=Odds ratio, CI=Confidence interval, VIF=Variance inflation factor. [a]Restricted to 922 daily low-dose GnRHa cycles with fresh embryo transfers, [b]Conducted with model-wise deletion, [c]With model-wise deletion, 731 observations were retained and 20.8% of the daily low-dose GnRHa cycles with embryo transfer were dropped due to incomplete information on any of the predictor variable. Chi-squared for Hosmer-Lemeshow Goodness-of-Fit test was 7.55 and *P* value was 0.52; C-statistic was 0.66. VIF ranged from 1.01 to 1.20 for all included covariates, [d]From the complete dataset, where missing data were imputed using multiple imputation with chained equations. All the 922 cycles were included. VIF ranged from 1.01 to 1.47 for all the included covariates. Definitions=1. Proportion of good quality embryos as observed on day 3 after insemination/total normally fertilized embryos per patient. **P*<0.05, †*P*≤0.01, ‡*P*≤0.001

Table 7: Summary of missing data[a]

Variable	Depot	Daily low-dose	Total
BMI	2088; 18 (0.9)	1283; 16 (1.2)	3371; 34 (1.0)
Types of infertility	2100; 6 (0.3)	1296; 3 (0.2)	3396; 9 (0.3)
Duration of infertility	2054; 52 (2.5)	1274; 25 (1.9)	3328; 77 (2.3)
Ovarian reserve			
Basal FSH, IU/L	2068, 38 (1.8)	1279; 20 (1.5)	3347; 58 (1.7)
Basal LH, IU/L	2068; 38 (1.8)	1279; 20 (1.5)	3347; 58 (1.7)
Basal E_2, pg/ml	2069; 37 (1.8)	1279; 20 (1.5)	3348; 57 (1.7)
Bilateral AFC	2086; 20 (1.0)	1266; 33 (2.5)	3352; 53 (1.6)
Duration of down-regulation[b]	2089; 0 (0)	1272; 2 (0.2)	3361; 2, (0.1)
LH after down-regulation	1766; 340 (16.9)	1097; 202 (15.6)	2863; 542 (15.9)
hCG day parameters[c]			
LH	2020; 69 (3.3)	1251; 23 (1.8)	3271; 92 (2.7)
E_2	1744; 345 (16.5)	1117; 157 (12.3)	2861; 502 (14.9)
P	2021; 68 (3.3)	1246; 28 (2.2)	3267; 96 (2.9)
Endometrium thickness	1992; 97 (4.6)	1200; 74 (5.8)	3192; 171 (5.1)

FSH=Follicle-stimulating hormone, LH=Luteinizing hormone, E_2=Estradiol, AFC=Antral follicular count, hCG=Human chorionic gonadotropin, BMI=Body mass index, P=Progesterone. [a]All are presented as number of available observations, number of missing observations (percentage of missingness); [b]Number of days from the first day of GnRHa down-regulation to ovulation trigger with hCG, excluding those whose ovum retrieval was cancelled; [c]Only for the 3363 cycles with ovum pick-up

12.3% missing in depot group versus daily low-dose group) [Table 7]. In the depot group, the significant association between OC and clinical pregnancy seen with model-wise deletion disappears when the regression model is rerun after MI [Table 4]. Similarly, the regression coefficients for LH on Gn initiation and serum P on hCG day, which are statistically significant under model-wise deletion, become nonsignificant

when the regression model is applied to the dataset with imputed data [Table 6]. Coefficients of the other variables in both models remained approximately the same after MI.

DISCUSSION

In this study, we accumulated clinical data for a large cohort for 1.0/1.3 mg depot and 0.05/0.1 mg daily luteal phase GnRHa protocols. Although the time frame was relatively long, all the clinical and embryological treatments were administered by the same group of physicians and embryologists, and the treatment protocols were stable during this period. With multivariable logistic regressions, we found that age, embryo quality and endometrial thickness on hCG day are important factors associated with clinical pregnancy for both of the two protocols. While previous literature identified similar prognostic factors for various COS protocols, the present study further elucidates the importance of these predictors for the two GnRHa protocol variants.[7,8,11]

In the present study, embryo transfer was canceled in all the cycles where P on hCG day was 1.5 ng/ml or above, which was based on previous studies on P and endometrial receptivity.[12-14] Most of the previous studies modeled serum P on hCG day as a dichotomous variable with the aim of identifying a prognostic cut-off; yet a linear relationship between P and pregnancy rate was shown below the thresholds.[15] Therefore, to fully utilize the information contained in the original data and further investigate these associations, we modeled P as a continuous variable. Interestingly, while higher serum P on hCG day and lower oocyte yield were negatively associated with clinical pregnancy in the depot group, such association was not observed in the daily low-dose group. Although the lack of such association in the daily low-dose group is consistent with previous reports, the negative association between P and clinical pregnancy rate in the depot group despite relatively low P (<1.5 ng/ml) seems counterintuitive.[12-14] Such discrepancy may be due to the difference of the ways in which P was modeled compared to previous studies, as categorizing variables may lead to loss of information and reduction in the likelihood of detecting a significant difference.[16] Also, patients in the two treatment groups differed in clinical characteristics, and such difference may also have contributed to the observed discrepancy, which needs to be further examined in prospective studies with adequate matching or randomization. Also, as previous studies have shown that the deleterious effect of premature progesterone rise could be circumvented by elective frozen embryo transfer, it will be interesting to compare the two groups regarding frozen-thawed cycle outcomes in the future.[17,18]

We found that embryo quality as assessed on day 3 is consistently associated with clinical pregnancy in both GnRHa groups. Similarly, according to a previous study quality and quantity of embryos are essential predictors of pregnancy outcomes; further, morphology-based embryo grading is not predictive for women ≥35 years.[11] Therefore, it may be necessary to separate the analyses for older and younger subgroups when statistical power is sufficient.

It was believed that excessive pituitary suppression by GnRHa may compromise ovarian response, embryo development and functions of the corpus luteum.[19,20] Although we have not observed any significant association between LH on hCG day and clinical pregnancy, such discrepancy may be explained by the "add-back" of recombinant LH in indicated cycles. We also found that serum LH on Gn initiation was not associated with clinical pregnancy in either group, which is consistent with results of a previous RCT.[21] However, the impact of LH could also be modified by age, which can be tested by future studies.[22]

Missingness is pervasive in clinical data, which is often directly or indirectly conditional on patients' characteristics or treatment outcomes.[9] Hence, by including only cases with complete data, researchers may introduce bias because the sample thus obtained is no longer based on a random selection from the source population. However, such complete case analysis approach is implicit in most of the previous clinical studies and methods used to address data missingness were rarely discussed. On the other hand, classical epidemiologic studies have proved that MI allows maximal utilization of the available information, takes the uncertainties during imputations into account, reduces bias and provides grossly correct estimates of standard errors.[9,10] In the present study, we observed that model-wise deletion (complete case analysis) gives rise to spurious associations between OC priming, serum *P* on hCG day, serum LH on Gn initiation day and clinical pregnancy, which disappear when analyses are repeated after MI. Although MI is still based on certain assumptions and may be different from the truth, it is still a valuable tool for sensitivity analysis that assesses the impact of data missingness and robustness of the study results.

There are a few limitations in this study. Firstly, as the two groups were unbalanced in baseline factors, confounding by indication is likely, and hence head-to-head comparison could not be made. However, in theory since the GnRHa preparation was given as one-third of the standard dose in the depot group, its ovarian suppression effect may be similar to that of the daily low-dose protocol, which awaits further evaluation in prospective studies. In addition, the data presented here may still be useful in counseling patients with similar demographic or clinical characteristics when they undergo treatments following these protocols.

Second, as a stepwise model selection approach was used, predictors in the final models may have been chosen by chance. Nevertheless, the predictor variables identified in this study have been consistent with previous reports.[7,8] Third, data missingness is significant in the present study. Yet, we have applied MI to investigate the impact of missing data on study results, which were not attempted by most of the previous outcome studies.

CONCLUSION

Age, embryo quality and endometrial thickness on hCG day are important prognostic factors for both the two GnRHa protocols; impacts of *P* on hCG day, oocyte yield, fertilization rate and number of cryopreserved embryos are less consistent. MI is valuable for sensitivity analysis that gauges robustness of the study results.

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