

[10.1071/RD21259](https://doi.org/10.1071/RD21259)

Reproduction, Fertility and Development

Supplementary Material

Timing considerations for removal of early cumulus cells in short-term insemination strategies

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Supplementary Table S1 Patient and cycle stimulation characteristics of the MPN rate=0% and MPN rate>0% groups

Characteristics	MPN rate=0% n=383, 50.93%	MPN rate>0% n=369, 49.07%	<i>P</i> -value
Female age	32.57±5.25	31.37±4.87	<i>P</i> =0.001 ^{*a}
Male age	34.42±6.05	33.49±5.88	<i>P</i> =0.032 ^{*a}
Female infertility type, n (%)			
primary infertility	243 (63.45)	243 (65.85)	
secondary infertility	140 (36.55)	126 (34.15)	<i>P</i> =0.490 ^c
Female infertility duration (years)	4.14±2.83	3.84±2.53	<i>P</i> =0.332 ^b
Female BMI	22.01±3.34	21.82±3.23	<i>P</i> =0.429 ^b
Male infertility type, n (%)			
primary infertility	270 (70.50)	276 (74.80)	
secondary infertility	113 (29.50)	93 (25.20)	<i>P</i> =0.186 ^c
Male infertility duration (years)	3.98±2.82	3.89±2.39	<i>P</i> =0.468 ^b
Male BMI	23.85±3.15	24.26±3.92	<i>P</i> =0.200 ^b
Duration of ovarian stimulation (days)	10.44±2.58	10.99±2.12	<i>P</i> =0.002 ^{*b}
Gonadotrophins per oocyte (ampoule ^d)	6.30±8.53	3.45±4.60	<i>P</i> <0.001 ^{*b}
Number of oocytes retrieval	10.82±7.78	16.05±8.99	<i>P</i> <0.001 ^{*b}
MII rate (%)	87.40±16.23	89.96±12.50	<i>P</i> =0.282 ^b
Ovarian stimulation protocol			
GnRH-agonist prolonged protocol	87 (22.72)	99 (26.83)	
GnRH-agonist long protocol	101 (26.37)	149 (40.38)	
GnRH-antagonist protocol	11 (2.87)	11 (2.98)	
Mini-stimulation protocol	96 (25.07)	66 (17.89)	
Luteal phase ovarian stimulation protocol	84 (21.93)	43 (11.65)	
Natural cycle	4 (1.04)	1 (0.27)	<i>P</i> <0.001 ^{*c}
Timing of early cumulus cells removal (h) ^e			

time \leq 4	20 (5.22)	28 (7.59)	
4<time \leq 4.5	90 (23.50)	85 (23.04)	
4.5<time \leq 5	140 (36.55)	135 (36.59)	
5<time \leq 5.5	72 (18.80)	87 (23.58)	
5.5<time \leq 6	34 (8.88)	17 (4.61)	
time>6	27 (7.05)	17 (4.61)	<i>P</i> =0.058 ^c

There were 752 cycles in our study, and each cycle had a MPN rate. Therefore, there were 752 MPN rates in our study. The median of these 752 MPN rates was 0.00%. Cycles were divided into two groups according to the median. The patient and cycle stimulation characteristics of the two groups are summarized in this table.

MPN: multiple pronuclei.

* Significant difference ($P<0.05$).

^aTwo-sample t-test was used for the data with homogeneous variance. Values are mean+SD.

^bTwo-sample Mann–Whitney test was used for the data with heterogeneous variance. Values are mean+SD.

^cPearson χ^2 test was used for categorical variables. Values are number (percentage).

^d75 IU per ampoule.

^eThe timing was defined as the time-interval between cumulus cells removal and insemination.

Supplementary Table S2 Binary logistic regression on multiple pronuclei rate

Predictors	OR(95% CI)	P-value
Female age	0.987 (0.934; 1.043)	0.646 ^a
Male age	1.027 (0.982; 1.074)	0.245 ^a
Duration of ovarian stimulation (days)	1.055 (0.972; 1.146)	0.203 ^a
Gonadotrophins per oocyte (ampoule °)	0.961 (0.926; 0.997)	0.035 ^{*a}
Number of oocytes retrieval	1.074 (1.044; 1.103)	<0.001 ^{*a}
MII rate	6.758 (2.136; 21.382)	0.001 ^{*a}
Ovarian stimulation protocol		
GnRH-agonist prolonged protocol	1	
GnRH-agonist long protocol	1.486 (0.985; 2.244)	0.059 ^b
GnRH-antagonist protocol	0.995 (0.377; 2.629)	0.992 ^b
Mini-stimulation protocol	1.611 (0.929; 2.796)	0.090 ^b
Luteal phase ovarian stimulation protocol		
Natural cycle	1.006 (0.087; 11.584)	0.996 ^b
Timing of early cumulus cells removal (h) ^d		
5.5<time≤6	1	
time≤4	4.568 (1.855; 11.245)	0.001 ^{*b}
4<time≤4.5	2.551 (1.257; 5.176)	0.009 ^{*b}
4.5<time≤5	2.270 (1.156; 4.456)	0.017 ^{*b}
5<time≤5.5	2.740 (1.351; 5.554)	0.005 ^{*b}
time>6	1.367 (0.551; 3.392)	0.500 ^b

Binary logistic regression was performed using to control for those factors with greater clinical importance and p values less than 0.1 in univariate analysis (Supplementary Table I). According to clinical experience, the fertilization of immature oocytes is easier to be abnormal. Therefore, the MII rate was included in the logistic regression although its p value is larger than 0.1 in Supplementary Table I. The GnRH-agonist prolonged protocol and 5.5<time≤6 categories were used as references.

Because more than half of MPN rates were 0.00% (abnormal distribution), we used binary logistic regression to predict the MPN rate, rather than multiple linear regression.

* Significant difference ($P<0.05$).

^a *P*-value of each variable's overall effects after adjusting for the other variables.

^b *P*-value between each variable's subgroups and reference group.

^c 75 IU per ampoule.

^d The timing was defined as the time-interval between cumulus cells removal and insemination.

Supplementary Table S3 Simple and multiple linear regressions on OPN without cleavage rate

Characteristics	Simple linear		Multiple linear	
	regression Coefficient	<i>P</i> -value	regression Coefficient	<i>P</i> -value
	95%CI		95%CI	
Female age	-0.005(-0.008; -0.002)	0.004*	-0.003(-0.009; 0.002)	0.249 ^a
Male age	-0.003(-0.005; 0.000)	0.058	0.002(-0.003; 0.006)	0.457 ^a
Female infertility type				
primary infertility	1			
secondary infertility	-0.022(-0.055; 0.011)	0.195		
Female infertility duration (years)	-0.002(-0.008; 0.004)	0.505		
Female BMI	0.000(-0.005; 0.005)	0.913		
Male infertility type				
primary infertility	1			
secondary infertility	-0.019(-0.054; 0.017)	0.309		
Male infertility duration (years)	-0.004(-0.010; 0.002)	0.242		
Male BMI	0.001(-0.004; 0.005)	0.826		
Ovarian stimulation duration (days)	0.010(0.003; 0.016)	0.005*	0.011(0.003; 0.019)	0.007 ^{*a}
Gonadotrophins per oocyte (ampoule °)	-0.005(-0.007; -0.003)	<0.001 *	-0.005(-0.008; -0.002)	0.001 ^{*a}
Number of oocytes retrieval	0.003(0.001; 0.005)	<0.001 *	0.000(-0.002; 0.002)	0.977 ^a
MII rate	-0.166(-0.275; -0.057)	0.003*	-0.135(-0.244; -0.026)	0.015 ^{*a}
Ovarian stimulation protocol				
GnRH-agonist prolonged protocol	1		1	
GnRH-agonist long protocol	-0.034(-0.077; 0.008)	0.110	-0.032(-0.074; 0.009)	0.127 ^b
GnRH-antagonist protocol	-0.023(-0.121; 0.075)	0.646	-0.003(-0.100; 0.094)	0.954 ^b
Mini-stimulation protocol	-0.065(-0.112; -0.018)	0.007*	-0.014(-0.069; 0.040)	0.609 ^b

Luteal phase ovarian stimulation protocol	-0.036(-0.086; 0.014)	0.157	0.010(-0.046; 0.066)	0.727 ^b
Natural cycle	-0.227(-0.424; -0.029)	0.024*	-0.090(-0.305; 0.126)	0.414 ^b
Timing of early cumulus cells removal (h) ^d	0.041(0.020; 0.061)	<0.001 *	0.034(0.013; 0.055)	0.001 ^{*a}

Multiple linear regression was performed using to control for those factors with p values less than 0.1 in univariate analysis. Categorical variables were transformed into dummy variables in these analyses. The dummy variables of each categorical variable in multiple linear regression were under the principle that “in and out together”. The primary infertility and GnRH-agonist prolonged protocol categories were used as references.

* Significant difference ($P < 0.05$).

^a P -value of each variable’s overall effects after adjusting for the other variables.

^b P -value between each variable’s subgroups and reference group.

^c 75 IU per ampoule.

^d The timing was defined as the time-interval between cumulus cells removal and insemination.

Supplementary Table S4 Simple and multiple linear regressions on grade 1–2 embryo rate at day 3

Characteristics	Simple linear		Multiple linear	
	regression Coefficient	<i>P</i> -value	regression Coefficient	<i>P</i> -value
	95%CI		95%CI	
Female age	0.008(0.004; 0.013)	<0.001*	0.003(-0.005; 0.010)	0.526 ^a
Male age	0.006(0.003; 0.010)	0.001*	0.001(-0.005; 0.008)	0.659 ^a
Female infertility type				
primary infertility	1			
secondary infertility	-0.006(-0.053; 0.041)	0.804		
Female infertility duration (years)	0.009(0.001; 0.018)	0.026*	0.006(-0.003; 0.014)	0.204 ^a
Female BMI	-0.003(-0.010; 0.004)	0.419		
Male infertility type				
primary infertility	1			
secondary infertility	0.036(-0.014; 0.086)	0.156		
Male infertility duration (years)	0.007(-0.002; 0.015)	0.135		
Male BMI	0.000(-0.007; 0.006)	0.886		
Ovarian stimulation duration (days)	-0.002(-0.012; 0.007)	0.621		
Gonadotrophins per oocyte (ampoule °)	0.007(0.003; 0.010)	<0.001*	0.004(0.000; 0.008)	0.032* ^a
Number of oocytes retrieval	-0.004(-0.006; -0.001)	0.003*	0.000(-0.003; 0.004)	0.946 ^a
MII rate	0.096(-0.058; 0.251)	0.220		
Ovarian stimulation protocol				
GnRH-agonist prolonged protocol	1		1	
GnRH-agonist long protocol	0.003(-0.056; 0.062)	0.927	0.002(-0.057; 0.061)	0.951 ^b
GnRH-antagonist protocol	0.057(-0.081; 0.194)	0.416	0.050(-0.087; 0.187)	0.472 ^b
Mini-stimulation protocol	0.066(0.001; 0.132)	0.048*	0.034(-0.041; 0.110)	0.372 ^b
Luteal phase ovarian stimulation	0.090(0.020; 0.160)	0.012*	0.045(-0.034; 0.123)	0.266 ^b

protocol				
Natural cycle	-0.028(-0.305; 0.248)	0.840	-0.062(-0.345; 0.221)	0.667 ^b
Timing of early cumulus cells removal (h) ^d	-0.037(-0.066; -0.008)	0.014*	-0.034(-0.063; -0.004)	0.024* ^a

Multiple linear regression was performed using to control for those factors with p values less than 0.1 in univariate analysis. Categorical variables were transformed into dummy variables in these analyses. The dummy variables of each categorical variable in multiple linear regression were under the principle that “in and out together”. The primary infertility and GnRH-agonist prolonged protocol categories were used as references.

* Significant difference ($P < 0.05$).

^a P -value of each variable’s overall effects after adjusting for the other variables.

^b P -value between each variable’s subgroups and reference group.

^c 75 IU per ampoule.

^d The timing was defined as the time-interval between cumulus cells removal and insemination.