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Follicle Aspirating is an Effective Remedy When Multi-follicles Developed in Donor-sperm Timing-Artificial Insemination

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Abstract

Purpose: To avoid multi-pregnancy, the cycle usually has to be cancelled when Multi-Follicles Developed (MFD) in artificial insemination. For the strong willing to continue the cycle for most patients, we explored the effectiveness of excess follicles aspirating as another remedy when multiple follicle developed.

Methods: We conducted a retrospective study in patients taking Artificial Insemination with Donor sperm (AID) and ovarian stimulation protocol from 2011 to 2022. Patients were divided in 4 groups according to the differences of receiving aspirating and follicle number. Clinical pregnancy rate, multi-pregnancy rate (twin pregnancy and high order pregnancy, separately) were mainly compared in our study.

Results: When multi-follicles developed, patients taking excess follicle aspirating achieved a comparable clinical pregnancy rate with those without aspirating (30.7% vs 26.1%). These two groups had a similar multi-pregnancy rate, 21.7% and 17.4% respectively, while high order pregnancy was rather lower in excess follicles aspirating group. MFD patients carried a significant higher clinical pregnancy rate, multi-pregnancy rate than patients with two dominate follicles patients under the age of 35. In ovarian stimulation protocol, patients with two dominate follicles carried nearly the same clinical pregnancy rate with one dominate follicle patients (21.4 vs 21.5%). Gemellary pregnancy rate was significant lower in one than two dominate follicles group (7.5% vs 0.4%) when patient's age was under 35. In the age of 35 or older, the clinical pregnancy rate and multi-pregnancy rate were similar in patients with multi-follicles and two dominate follicles.

Conclusion: In AID, in the age of lower than 35, when multi-follicles developed, excess follicle aspirating with two dominate follicles reserved effectively deceased high order pregnancy and ensured the clinical pregnancy rate at the same time. From the perspective of singleton, it was feasible to keep one dominate follicle reserved.

Keywords: AID; Multi-follicle development; Multi-pregnancy rate; Follicle aspirating

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Introduction

For the superiority of low cost and less invasive, Artificial Insemination (AI) is an easy way widely used in infertile couples [1,2]. It is the first choice for infertile couples with male factors such as sexual dysfunction or azoospermatism [3,4]. To ensure pregnancy, the basic essentials are 1. sufficent quantity and quality sperms [5] 2. At least one maturing follicle development and ovulation [6] 3. Proper time and conditions for fertilization [7]. As we known, the quantity and quality of female eggs decrease with age. It was reported that there was only one out of three eggs with high quality [8]. It was commonly accepted for both physicians and patients to achieved more than one follicle by ovulation induction for the aim of getting pregnancy soon. Ovulation induction used to apply to patients with ovulation dysfunction, researchers suggested that it increased clinical pregnancy rate in unexplained infertility patients with normal ovulation [9,10]. However, despite of the influence in clinical pregnancy rate, multi-follicles may lead to the increase of multi-pregnancy.

Multi-pregnancy caused by MFD was the major complication in ovulation induction. It was reported the rate of multiple gestation was 20 to 100 times higher in ovarian stimulation cycles than in nature cycles [11]. As reported, multi-pregnancy, especially high order multiple pregnancy, lead to adverse obstetric outcomes [12,13]. Though, fetal reduction was a measure to improve the outcomes of multi-pregnancy, it didn't total reverse the undesirable outcomes [14].

Thus, the prevention of multi-pregnancy, especially high order pregnancy becomes more important. Usually, the cycle had to be cancelled when MFD occurred in AI. It was hardships for patients to cancel the cycle, especially for the aged or patients with ovulation dysfunction. For this reason, follicle aspirating as a remedy for MFD in artificial insemination has become increasingly valued. Follicle aspirating was first reported by *Christian* in 1998 [15]. A few years later, the ASRM suggested it was considerable to aspirate excessive follicles after administration of HCG in 2006 [16]. Regretfully, few researchers reported the details of this measure. It still remained unclear about the effectiveness and the suitable reserved follicle number in excessive follicles aspirating. Thus, we conducted a retrospective study to reveal the effectiveness of extra follicle aspirating and explore the suitable reserved follicle number so as to afford another choice for patients. The clinical pregnancy rate and multi-pregnancy rate were the primary outcomes. We also made a comparison about one and two follicles in donor-sperm artificial insemination as a guidance for the number of follicle reserved in follicle aspirating.

Materials and Methods

We analyzed patients whose oviducts were both unobstructed, taking ovarian stimulation protocol and undergoing Donor-sperm artificial insemination from 2011 to 2022 in reproductive center, Guangdong Provincial Fertility Hospital.

Ovarian Stimulation

The ovarian stimulation protocol included oral drugs (Clomiphene, Letrozole) or Gonadotrophin(Gn) alone and the two combined. Ovarian stimulation was started from day 2 to 4 of menstrual cycle. Follicle development was monitored by transvaginal ultrasonography and Gn dose adjusted every 1 to 3 days by physician's experience if necessary. The urine Luteinizing Hormone (LH) test paper was tested when the leading follicle reached average diameter of 16mm, there after, serum LH and progesterone were measured when necessary.

Human Chorionic Gonadotropin (HCG) Trigger

The administration of HCG was immediately when the test paper was positive or the leading follicle reached average diameter of 20mm. The dosages of HCG were around 6000-10000iu accordingly.

Follicle Aspirating

From 2020, once there were 3 dominating follicles (average diameter ≥14mm) development; follicle aspirating was conducted after the administration of HCG so that only 2 dominate follicles were reserved. Physicians would try their best to ensure one dominate follicle for each ovary if possible. The concrete operation was similar as oocyte retrieval. The excess follicles (average diameter ≥12mm) were aspirated and abandoned in the operation under the guidance of ultrasound.

Artificial Insemination

The timing artificial insemination was carried out according to the time of HCG administration and serum LH level. To our experience, insemination performed nearly before and after ovulation achieved better results. The sperms were provided by human sperm bank in Guangdong Province.

Luteal Support

Luteal support was started from the first day after ovulation. Patients took up to 400mg progesterone daily for 14 days. Once the pregnancy was confirmed, luteal support went on.

Pregnancy Confirmation

A blood test was drawn to confirm pregnancy for patients. We viewed a uterine pregnancy with babies' heart as clinical pregnancy.

Basis for group

According to the difference of dominate follicle's number and whether follicle aspirating was taken, patients were divided into 4 Groups. In group A, there were more than 3 dominate follicles developed in which follicle aspirating were taken while did not in group B. Group C and D included patients with no more than 2 dominate follicles taking ovarian stimulation protocol, one dominate follicle for Group C and 2 for Group D.

Statistical Analysis

Statistical analyses were performed using SPSS software (version 21.0 for Windows®;). Student's t-test was used for continuous variables and chi-square tests for categorical variables. Continuous variables are presented as mean±SD, categorical variables are presented as rate (%). Two tailed tests were employed, and $P<0.05$ was considered to indicate statistical significance.

Results

We searched 36267 cycles in the database in our center from 2011 to 2022, a total of 11240 cycles were analyzed in our study.

There were no significant difference in age, basic-FSH level, basic-LH level, endometrial thickness with Group A and Group B. Sperm parameters was significantly better in Group B than in Group A, while, unexpected, the clinical pregnancy rate was comparable in this two groups (30.7% vs 26.1%, $p>0.05$). With

Table 1: The comparison of basic characteristics and outcomes of Groups.

Characteristics	Group A n=75	Group B n=1645	Group C n=6886	Group D n=2634	P value for A&B	P value for C&D	P value for B&D
Baseline for maternity							
Age (year)	29.47±3.52	29.33±3.97	29.50±4.06	29.63±4.07	0.778	0.164	0.523
bFSH (mIU/ml)	6.45±1.52	6.40±1.92	7.19±1.97	6.30±1.95	0.826	0.485	0.103
bLH (mIU/ml)	5.74±2.89	4.82±2.62	4.97±2.42	5.70±3.26	0.212	0.118	0.057
Endometrial thickness(mm) in the first AI	10.09±1.91	11.10±1.89	10.72±1.84	10.79±1.87	0.219	0.106	0.614
Sperm parameter in the first time of AI(Before ovulation)							
Concentration (million/ml)	57.76±13.09	62.44±22.55	61.21±15.47	61.66±22.30	0.075	0.346	0.266
PR (%)	47.65±3.86	52.01±7.99	50.86±13.09	50.50±6.82	0.000	0.176	0.000
Sperm parameter in the second time of AI(After ovulation)							
Concentration (million/ml)	73.40±21.03	63.49±18.89	64.36±18.56	65.76±19.44	0.000	0.001	0.000
PR[a] (%)	48.44±5.14	51.27±7.76	50.33±6.81	50.28±6.89	0.000	0.734	0.000
Primary cycle outcomes							
CPR[b] (%)	30.7%(23)	26.1%(429)	21.4%(1470)	21.5%(564)	0.381	0.492	0.007
MPR[c] (%)	21.7%(5)	17.4%(75)	0.4%(6)	8.0%(45)	0.577	0.000	0.000
Twins (%)	21.7%(5)	13.1%(56)	0.4%(6)	7.6%(43)	0.231	0.000	0.000
H-MPR[d] (%)	0	4.4%(19)	0	0.4%(2)	0.614	0.077	0.000

A: Progressive motile sperm; **B:** Clinical pregnancy rate; **C:** Multi-pregnancy rate; **D:** High order multi-pregnancy rate

Table 2: The comparison of basic characteristics and outcomes of Groups for the age of 35 lower.

Characteristics	Group A N=68	Group B N=1461	Group C N=6051	Group D N=2325	p value for A&B	P value for B&D	P value for C&D
Baseline for maternity							
Age (year)	28.75±2.79	28.40±3.10	28.47±3.08	28.63±3.10	0.368	0.717	0.706
bFSH (mIU/ml)	6.42±1.55	6.35±1.87	6.29±2.40	6.25±1.93	0.781	0.118	0.475
bLH (mIU/ml)	5.85±2.99	4.91±2.71	5.07±10.01	5.88±24.73	0.053	0.134	0.126
Endometrial thickness (mm) in the first AI	10.08±1.97	11.10±1.88	10.75±1.82	10.77±1.86	0.477	0.855	0.624
Sperm parameter in the first time of AI(Before ovulation)							
Concentration (million/ml)	57.57±13.52	62.48±23.16	61.23±15.49	61.74±23.11	0.084	0.343	0.322
PR (%)	47.49±3.88	51.95±8.03	50.86±13.68	50.37±6.73	0.000	0.000	0.097
Sperm parameter in the second time of AI(After ovulation)							
Concentration (million/ml)	72.50±20.99	63.16±18.67	64.33±13.58	65.77±19.05	0.000	0.000	0.002
PR (%)	48.10±4.36	51.16±7.67	50.33±6.82	50.22±6.87	0.000	0.000	0.498
Primary cycle outcomes							
CPR (%)	30.9%(21)	26.0%(380)	21.4%(1294)	21.6%(503)	0.398	0.002	0.788
MPR (%)	19.0%(4)	18.7%(71)	0.5%(6)	8.5%(43)	0.958	0.000	0.000
Twins (%)	19.0%(4)	13.7%(52)	0.5%(6)	8.2(41)	0.513	0.007	0.000
H-MPR (%)	0	5.0%(19)	0	0.4%(2)	0.613	0.000	0.079

regard to multi-pregnancy, though multi-pregnancy rate were similar (21.7% vs 17.4%, p>0.05), there was no triplet or greater pregnancy in Group A while 19 cases (4.4%) of triplet or greater pregnancy in Group B. All the multi-pregnancy cases in Group A were twins (Table 1).

The basic characteristics were similar in Group C and D. Clinical pregnancy rate was similar while multi-pregnancy rate was significant higher in Group C than in Group D (8% vs 0.4%, p=0.000). For there was no high order pregnancy in Group A, we analyzed Group B and Group D. The statistics showed the baseline were similar in the two groups. The clinical pregnancy rate, multi-pregnancy rate, high order pregnancy rate were significant higher in Group B than in Group D (Table 1).

We further compared those groups stratified by age. In the age of under 35, the outcomes were similar in Group A and Group B. Patients in Group B carried significant higher clinical

Table 3: The comparison of basic characteristics and outcomes of Groups for the age 35 or older.

Characteristics	Group A N=7	GroupB N=184	Group C N=835	Group D N=309	P value for B&D	P value for C&D	P value For B&C
Baseline for maternity							
Age (year)	36.43±1.90	36.73±1.77	36.97±2.04	37.17±2.11	0.130	0.140	0.138
bFSH (mIU/ml)	6.79±1.12	6.80±2.25	6.77±4.02	6.68±2.04	0.552	0.510	0.607
bLH (mIU/ml)	4.59±1.03	4.16±1.69	4.20±2.44	4.31±2.33	0.397	0.482	0.828
Endometrial thickness (mm) in the first AI	10.19±1.22	11.16±1.97	10.53±1.95	10.94±2.00	0.228	0.668	0.386
Sperm parameter							
Concentrition1	59.57±8.14	62.13±16.65	61.07±15.31	60.99±14.88	0.433	0.937	0.404
PR1	49.29±3.40	52.51±7.70	50.89±7.50	51.50±7.38	0.149	0.217	0.008
Concentrition2	82.14±20.86	66.27±20.45	64.60±18.41	65.70±18.92	0.187	0.375	0.278
PR2	51.71±10.01	52.17±8.40	50.34±6.75	50.73±7.02	0.053	0.383	0.006
Primary cycle outcomes							
CPR	---(2)	26.6%(49)	21.1%(176)	19.7%(61)	0.076	0.595	0.107
MPR	---(1)	8.2%(4)	0%	3.3%(2)	0.404	0.063	0.002
Twins	---(1)	8.2%(4)	0%	3.3%(2)	0.404	0.063	0.002
H-MPR	0	0%	0%	0%	-----	-------	----

pregnancy rate, multi-pregnancy rate and high order pregnancy rate than patients in Group D. Clinical pregnancy rate was similar in Group C and D while the multi-pregnancy rate was significant higher in patients in Group D (Table 2). When the age reached 35 or older, there were no significant difference in clinical pregnancy rate in Group B, Group C and Group D. Multi-pregnancy was higher in Group B than in Group C, while it was similar in Group B and Group D (Table 3). No high order pregnancy occurred in patients older than 35 years old. For the insufficient case (only 7 cases) in Group A, we didn't compare patients aged over 35 in this Group.

Discussion

In our study, we verified the effectiveness of excess follicle aspirating as a remedy for MFD. Excessive follicle aspirating achieved a comparable clinical pregnancy rate with MFD-patients without aspirating. When two follicles reserved from excessive follicle aspirating, the multi-pregnancy rate was similar while high order pregnancy largely decreased. Multi-pregnancy rate increased significantly with the increased follicle number in patients under 35 years old, and turned to be similar when the age reached 35 or older.

When we talked about MDF, there came a question that what were the detail diameter of dominate follicles? Researchers hold a view much alike. A similar research in China chosen average diameter of 14mm as dominate follicle [17]. In Intra-Uterine Insemination (IUI) cycles, *Teramoto* reported the follicles (from 12-14mm) on trigger day were associated with multi-pregnancy rate [18]. Study from *Scalici* regarded the number of follicle (from 12-15mm) as an independent and significant risk of multi-pregnancy rate [19]. Identically, most studies defined a mature follicle as the average diameter of over 14mm [20,21]. In our centre, we followed the mainstream idea and viewed the average diameter of over 14mm as dominate follicles. Follicles of over 12mm were taken into consideration to aspirate out when taking excessive follicle aspirating to avoid high order multi-pregnancy.

Many studies attributed multi-pregnancy much too ovarian induction [22-24]. Multiple pregnancies are also the major and the most serious iatrogenic complication associated with ovarian stimulation, which in return limited the use of this protocol. Studies confirmed that perinatal mortality rate were increased by 4 and 6 times in gemellary and triple pregnancy, respectively [25,26]. Besides, it was acknowledged that multi-pregnancy increased perinatal complications such as gestational diabetes mellitus, hypertensive disorder complicating pregnancy, premature delivery and so on, those adverse events greatly burdened patients and society [27,18]. Correlation has been reported between follicle number and multi-pregnancy rate in many researches [6,16]. Studies before also suggested the follicle number was predictive for multi-pregnancy [16,29]. In our center, almost triple or greater pregnancies were attributed to multi-follicle development in the last 10 years. In fact, it was not easy to totally avoid multi-follicle development. Researchers worked a lot to pursue a higher clinical pregnancy rate and a lower multi-pregnancy rate. It is basically essential for physicians to take measures to avoid high order pregnancy rate, ensure clinical pregnancy rate and reduce cycle cancellation simultaneously when multi-follicles developed. To our study, follicle aspirating makes the best of both worlds.

In this study, when taking follicle aspirating, MFD patients reached a better clinical pregnancy of 30.7% compared with MFD-patients without aspirating though there was no statistically significance. It was not clear why there was a rising tendency in clinical pregnancy after taking follicle aspirating. We speculated that it may be related to more accurately operating time nearly to ovulation in follicle aspirating group. As commonly reported, MFD patients achieved a better clinical pregnancy rate as two dominate follicle group under the age of 35. When patients reached the age of 35 or older, clinical pregnancy was not improved by the increased follicles. The data suggested the influence of MFD was largely related to maternal age. We didn't pay much attention to the difference of sperm for the reason that all the sperms were ensured qualified to artificial insemination by sperm bank. A study from a 21 years experience also

suggested the clinical pregnancy rate has little to do with sperm count in artificial insemination [30].

It was reported that twin pregnancy rate was of 15-20% and high order pregnancy rate was of about 5% in artificial insemination [31,32], which were close to data in our center. In our research, excess follicle aspirating reduced high order pregnancy rate from 4.4% to nearly 0, instead of a higher twin pregnancy rate of 21.4%. Due to the small sample size, no high order pregnancy occurred in aspirating group. To further verified the effectiveness of excessive follicle aspirating in reducing high order pregnancy rate, we tried to instead follicle aspirating group by two dominate follicles group as they had the same number of dominate follicles reserved. A further comparison conducted between MFD without aspirating and two dominate follicles group has demonstrated the multi-pregnancy rate and high order pregnancy rate were largely declined when follicle number decreased to two in patients under the age of 35. The difference was not found when patients' age reached 35 or older. The results from comparison stratified by age in our study suggested age was a vital factor to cycle success and multi-pregnancy rate, as found by *Immediata* in 2020 [30]. Thus, by the date above, we considered follicle aspirating largely decreased the risk of high order pregnancy and fetal reduction subsequently through reducing the number of follicles.

A fact should not be ignored was the higher twin pregnancy rate after excess follicle aspirating. Although, with the progress of fetal medicine and obstetrics, twins were more acceptable by obstetricians and families. It was also necessary to avert gemellary pregnancy since there were still high preterm rate and complications in fetal and pregnant woman compared with singleton pregnancy. Recent years, a growing number of researchers explored the outcomes of fetal reductions from twins to singletons. The studies showed a fetal reduction improved the perinatal outcomes of dichorionic diamniotic twins [33]. Hence, how many follicles are properly to be reserved? ASRM suggested no more than 2 mature follicles was appropriate for women with polycystic ovarian syndrome and hypothalamic an ovulation, while more than 2 mature follicles for women with unexplained or age-related infertility [16]. Early research attached to taking follicle aspirating with 3 dominate follicle left [15], in their study, there were 3 triple-pregnancies and 2 cases of 4-5 fetal pregnancies which finally conducted fetal reduction. In our study, from the perspective of making best use of follicles and patient's strong willingness, two follicles were reserved. An analyze of one and two dominate follicles development in ovarian stimulation cycles in our study showed that they got quite similar clinical pregnancy rate, while a significant higher twin pregnancy rate in two dominate follicles group (7.5% vs 0.4%). Comparison stratified by age showed that the difference in multi-pregnancy rate was distinct in the age under 35. A study from China got quite similar results [17]. The increased one follicle contributed much to multi-pregnancy rather than clinical pregnancy in young women. From the perspective of singleton pregnancy, one dominate follicle may be more suitable for donor artificial insemination to our study. When there was a need of excess follicles aspirating, it may be safer to keep one dominate follicle left, especially in patients under the age of 35. For patients older than 35, as there were no high order pregnancy occurred and gemellary pregnancy rate was rather low in our study, we suggested whether there was a need to take follicle aspirating depended on individual conditions.

Limitations

Failure to divide the reason of ovarian stimulation and the insufficient samples in aspirating group were the major limitations of our study. With the data increasing, it is possible to design a subgroup analysis basing on subjects such as treatment cycle, menstrual cycle in later research. For the high rate of twin pregnancy with two dominate left, it is worth to explore one dominate follicle reserved when taking follicle aspirating.

Conclusion

In conclusion, in the age of lower than 35, excess follicle aspirating with two dominate follicles reserved ensured a considerable clinical pregnancy rate and a rather low high order multi-pregnancy rate in AID. From the perspective of singleton, it was feasible to keep one dominate reserved. Despite the availability of follicle aspirating, avoiding multi-follicle development remains a top priority for doctors.

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