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The effect of bromocriptine-rebound method on ongoing pregnancy and live birth after intracytoplasmic sperm injection cycles: a randomized clinical trial

Ashraf Alleyassin MD, Ramak Esmailizad MD, Marzieh Agha-Hosseini MD, Leila Safdarian MD, Fatemeh Sarvi MD, Abas Kokab PhD

Department of Infertility, Tehran University of Medical Sciences, Tehran, Iran.

ABSTRACT

Purpose: To assess whether bromocriptine-rebound method (BRM) can improve pregnancy outcomes compared to long protocol after intracytoplasmic sperm injection cycles (ICSIs).

Materials and Methods: A total of 114 women underwent ICSI. Pregnancy outcomes and hormonal data were compared between two groups, i.e. long protocol and BRM. Ovulatory women with normal serum prolactin levels were assigned to either BRM (n = 57 cycles) or long protocol (n = 57 cycles). Both procedures were carried out in a similar way. However, a group of patients were given bromocriptine daily from the 4th day of the preceding cycle until 7 days before gonadotropin stimulation.

Results: There were no significant differences in the numbers of developed follicles,total retrieval oocytes, transferredembryo and embryos with superior morphology between the two groups. Also, the values of chemical, clinical and ongoing pregnancies and live births were not significantly different (36.8%, 35.1%, 28.1%, 28.1% in BRM group and 43.9%, 38.6%, 21.1% and 19.3% in long protocol, respectively). Ongoing pregnancy and live birth were significantly higher in chemical pregnancy in the BRM group (P = .04 and P = .035, respectively).

Conclusion: This prospective study demonstrated that BRM might lead to higher ongoing pregnancy and live birth rates compared to the long protocol in women undergoing intracytoplasmic sperm injection cycles.

Keywords: bromocriptine; intracytoplasmic sperm injection; live birth; prolactin; Iran.

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INTRODUCTION

Assisted reproductive technology (ART) has a significant role in treating infertile couples with broad range of causes. Despite its advances, the success of individual cycle to a live birth is still low.[1] This can be attributed to poor response to ovulation induction or implantation failure.[2,3] Different protocols have been recommended to optimize follicular development. However, the best protocol to improve ovarian stimulation has yet to be determined.

Several studies have suggested that prolactin has an important role in regulating all ovarian functions.[4,5] For instance, Kiapekou and colleagues assessed different isoforms of prolactin receptors in ovaries of mice. Their investigations revealed that the prolactin pathway is active in preantral follicles and prolactin induces oocytes maturation, fertilization and early embryonic development.[6] Also, Ozaki and colleagues showed that mid-luteal serum prolactin levels were significantly lower in patients with early pregnancy loss.[7] In addition, Oda and colleagues have shown that higher mean of serum prolactin concentrations before the oocyte retrieval increase the fertilization and cleavage rates compared to euprolactinemic and hypoprolactinemic cycles.[5]

Jinno and colleagues introduced a novel method based on rebound phenomena after bromocriptine administration.[8] They reported that this protocol can boost the response after ovarian stimulation leading to good quality oocytes. In this protocol, women with normal serum prolactin levels were recruited. Bromocriptine 2.5 mg per day was orally administered from day 5 of the preceding cycle until 7 days before ovarian stimulation. After that ovarian stimulation similar to long protocol was started. They concluded that oocyte maturation improvement in patients may be due to restoring post receptor responsiveness of granulose cells to prolactin during the hypoprolactinemic period and increasing of the prolactin concentration by a rebound phenomenon after discontinuation of bromocriptine.

Very limited number of researches has been published in assessing this protocol. Although this protocol seems to be promising, its effectiveness is still to be evaluated. The current study intended to specifically address the oocytes and embryo quality, pregnancy rate, ongoing pregnancy and live birth following bromocriptine-rebound method (BRM).

MATERIALS AND METHODS

This was a prospective controlled randomized clinical trial involving 114 volunteer patients attending our outpatient clinic in an infertility centerin Tehran. The patients were chosen between March 2009 and August 2010. The ethical approval of this study was obtained from the medical ethics committee of Tehran University of Medical Sciences. The patients received written information about the study and potential complications of assisted reproductive technology, followed by an oral discussion and a formal consent. The outcomes of intracytoplasmic sperm injection (ICSI) and hormonal therapy were compared between 57 patients on long standard protocol and 57 patients on BRM.

The patients who were enrolled in the study had normal hormonal screen in days 2-4 of cycle for follicle stimulating hormone (FSH)(<10 IU/mL), luteinizing hormone (LH), estradiol, thyroid-stimulating hormone (TSH)and prolactin. In addition, they were younger than 40 years old and their ovulatory cycle was determined by serum progesterone in late secretary phase**.** Women with ovulatory cycles were excluded from this study. Patients with severe male factor in sperm analyses according to WHO criteria were also excluded.[9]

The patients were randomly assigned to receive BRM or standard long protocol, the gonadotropin releasing hormone (GnRH) agonist and recombinant FSH (rFSH) regimen for ovarian stimulation. The randomization was based on computer-generated numbers in 19 blocks of 6 that were concealed until the time of interventions. Both the patients and the clinicians were aware of the allocation.

All patients of this study were treated with long protocol for ovarian stimulation. Oral contraceptive pill was started for pituitary down-regulation from day 3 preceding cycle. Patients were treated with daily administration of 0.5 mg buserelin acetate (Superfact, Aventis, Frankfurt, Germany) from day 21 of menstrual cycle. Buserelin acetate was reduced to 0.25 mg daily when ovaries were suppressed based on ultrasound, and ovarian hyperstimulation was initiated with recombinant follicle stimulating factor (Gonal F, Serono, Geneva, Switzerland) in different doses according to ovarian antral follicle count in ultrasonography on day 2 of withdrawal bleeding.

In the BRM group, bromocriptine (2.5 mg/day, Tehran, Iran, Aburaihan) was administered orally daily from day 3 of the preceding cycle until 7 days before ovarian stimulation. Serial ultrasound examinations were done to assess ovarian response, and then gonadotropin dose adjustments were done as required. Human chorionic gonadotropin (HCG) (Pregnyl, Organon, Oss, The Netherlands) 10,000 IU was administered when at least two follicles reached a mean diameter of 18 mm. Oocytes retrieval was performed 36 hours after HCG administration and oocytes were inseminated with ICSI. Embryo transfer was performed on the 2nd day. Luteal support with progesterone in oil (Progesterone, Aburaihan Co., Tehran, Iran) 25 mg daily as intramuscular injection and vaginal suppository Cyclogest (Cox Pharmaceutical, Barnstaple, UK) was started on the day of oocytes retrieval. The documentation of fetal heart activity on ultrasound was continued. The ART cycles outcome measures were live birth rate (delivery of gestation) and clinical pregnancy rate (an intrauterine gestational sac by ultrasound). ICSI outcomes and hormonal data were compared between the two groups to examine clinical efficacy of the BRM.

Statistical analysis

The data were analyzed with the Statistical Package for the Social Sciences version 17 (SPSS Inc, Chicago, IL, USA). The normality of quantitative data was checked by histogram and one sample Kolmogorov-Smirnov test. Because of the non-parametric distribution of continuous data, Mann–Whitney *U* test was used to compare the data of the two groups. Qualitative variables were compared

with chi-square or Fisher exact tests when appropriate. Two tailed $P < .05$ was considered as significant.

RESULTS

No significant differences were observed between BRM and long protocol groups in respect to mean age, body mass index, infertility duration and primary or secondary infertility (Table 1). The serum FSH, LH, TSH, estradiol and prolactin levels also demonstrated no significant differences between the two groups (Table 2). There were no significant differences between the two groups regarding the number of developed follicles, total retrieval oocytes, transferred embryos, and embryos with superior morphology (Table 1).

The chemical pregnancy rates were 36.8% and 43.9% in the intervention and control group, respectively, which is not statistically significant ($P = .445$) and led to clinical pregnancy in 35.1% of intervention participants and 38.6% of control participants ($P = .698$). Ongoing pregnancy occurred in 28.1% and 21.1% of the participants ($P = .384$) of the intervention and control group, respectively. All of the ongoing pregnancies led to live birth except one in the control group due to fetal anomaly ($P = .271$).

The rate of abortion was lower in the intervention group, i.e. 23.8%, compared to 52% in the control group which was statistically significant ($P = .051$). More women who achieved chemical proven pregnancy on the

Table 1. Patient and cycle characteristics and pregnancy outcomes of both studied groups (BRM and long protocol).

Parameters	BRM Group (n=57)	Long Protocol Group (n=57)	*P* Value
Age (years)[a]	28.7 ± 4.4	29.8 ± 4.5	.209
Body mass index (kg/m^2)[a]	26.8 ± 3.2	26.9 ± 4	.834
Duration of infertility (years)[a]	7.3 ± 4.4	7.2 ± 4.6	.963
Primary infertility (%)	89.1	83.3	.553
Number of previous ART cycles [b]	0 (0-1)	0 (0-2)	.104
Total dose of gonadotropins [a,c]	36.5 ± 17.6	38.4 ± 20.7	.604
E_2 day of human chorionic gonadotropin (pg/mL)[a]	3546.9 ± 5100.8	2529.1 ± 3331.8	.301
Number of retrieved oocytes [a]	12.4 ± 5.8	12.3 ± 6.5	.932
Number of metaphase II oocytes [a]	7.8 ± 4.0	7.9 ± 4.5	.932
Number of pronucleus [a]	6.0 ± 3.4	6.3 ± 3.5	.684
Total of embryo transfer [a]	3.1 ± 1.2	3.3 ± 1.1	.274
Number of top-quality embryo transfer [a]	4.6 ± 2.7	4.1 ± 2.5	.281
Freezed embryo [a]	2.0 ± 2.9	1.6 ± 2.3	.505
Fertilization rate (%)	76.7	79.4	.325
Implantation rates (%)	16.3	11.5	.196
Ovarian hyperstimulation syndrome (%)	7.0	3.5	.679
Chemical pregnancy rate (%)	36.8	43.9	.445
Clinical pregnancy rate (%)	35.1	38.6	.698
Ongoing pregnancy rate (%)	28.1	21.1	.384
Live birth rate (%)	28.1	19.3	.271
Multiple pregnancy (%)	31.3	16.7	.661
Miscarriage (%)	23.8	52.0	.051

Keys: E_2, serum estradiol; ART, assisted reproductive technology; BRM, bromocriptine-rebound method.
[a] values are mean ± SD
[b] value is median (range)
[c] No. of 75 IU injections

Table 2. Basic laboratory findings among patients in both BRM and long protocol groups.*

Mean Serum Level	BRM group (n = 57)	Long Protocol Group (n = 57)	P Value
Prolactin (ng/mL)	14.2 ± 8.6	16.0 ± 5.8	.617
Follicle stimulating hormone (mIU/mL)	6.9 ± 3	6.4 ± 2.7	.415
Luteinizing hormone (mIU/mL)	5.9 ± 3.7	5.7 ± 4.2	.846
Progestrone (ng/mL)	9.9 ± 9.7	13.5 ± 14.4	.345
Estradiol (pg/mL)	64.8 ± 70.2	58.3 ± 68.4	.705

* Data are presented as mean ± SD.

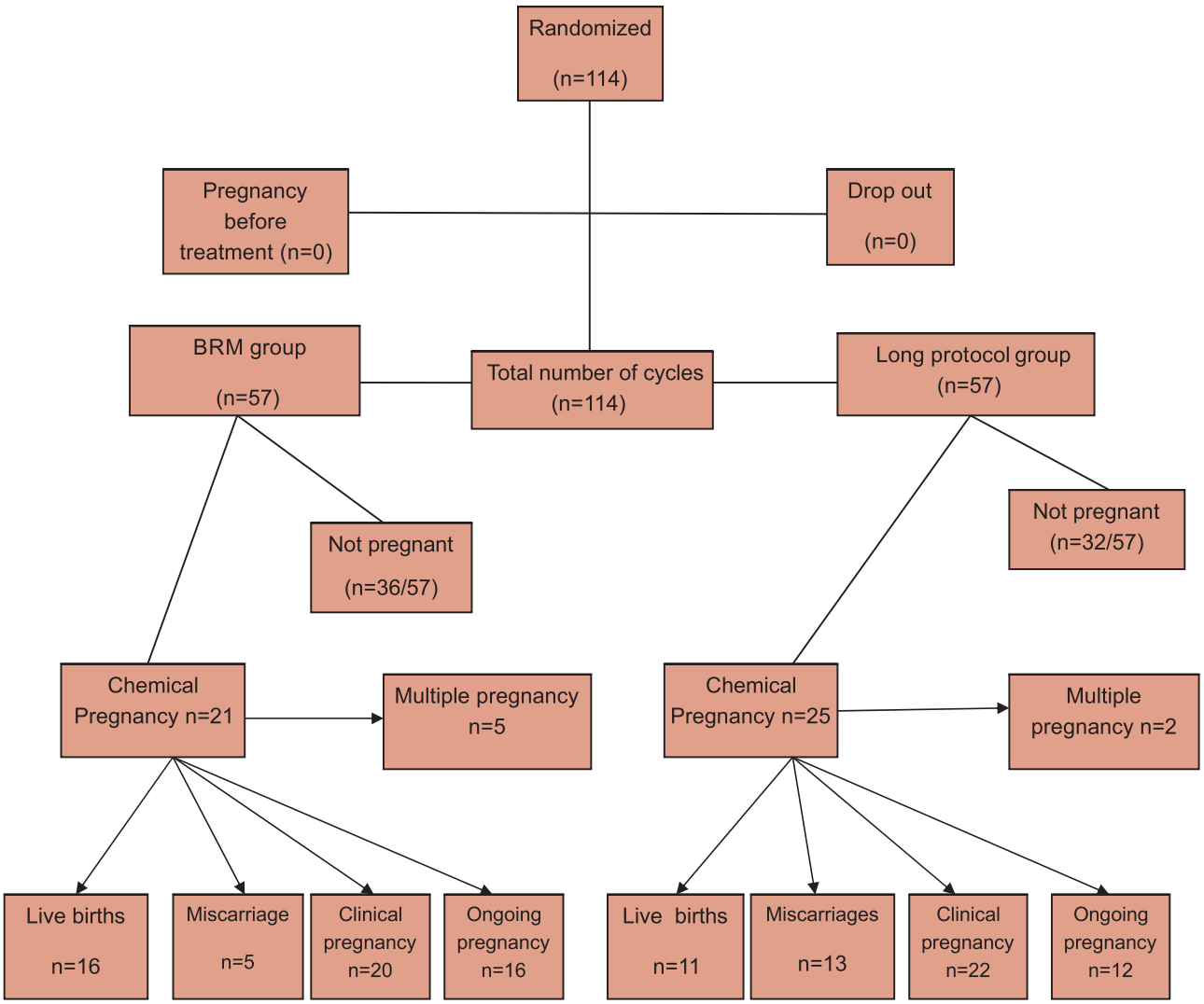

Figure. Flowchart of study.

long protocol ended their pregnancy due to miscarriage compared to the other group. On the other hand, the live birth rate was higher in intervention group, i.e. 28.1%, compared to 19.3% in the control group which was not significant ($P = .271$).

Five out of 16 (31.3%) live births in the intervention group and 2 out of 11 (18.2%) live births in the control group were twins ($P = .661$).Finally, women on long protocol who achieved chemical pregnancy had more miscarriage compared to BRM. It is of surprise that in 7% of women receiving BRM, the clinical manifestation of ovarian hyperstimulation syndrome was observed. This state in women receiving long protocol was 3.5%.

DISCUSSION

To the authors' knowledge, the current study is the first to support the results of a prospective study on women using BRM. In a prospective randomized trial, Jinno and colleagues compared in vitro fertilization (IVF) cycles outcome between patients on BRM and long protocol using GnRH agonist in women with previous IVF failure.[10] Their study demonstrated that patients on BRM had higher number of follicles, fertilized oocytes and embryos with top quality morphology. They concluded that clinical pregnancy rates and live birth rates per cycles were higher in women on BRM.

In another study, again Jinno and colleagues compared IVF outcomes between three groups: long protocol, bromocriptine-rebound regimen and bromocriptine-continuous regimen. Their results demonstrated that higher fertilization rates, cleavage rates, proportion of good quality embryos and pregnancy rates per oocytes retrieval were higher in BRM.[8] Also, they designed another research to answer why BRM improves IVF

outcomes.[11] In that study they described an increase in serum prolactin concentration and prolactin post receptor mRNA of granulose cells in patients with poor response to ovulation induction. During BRM, there is recovery of post receptor response during hypoprolactinemia period due to bromocriptine administration and increasing of serum prolactin levels as a rebound after bromocriptine discontinuation.

In our study, there was no significant differences in good quality retrieved oocytes and embryos with superior morphology between participants in BRM and long protocol groups. In our prospective randomized clinical trial, while chemical, clinical and ongoing pregnancy rates were equal in both BRM and long protocol groups, comparing these two groups in chemical pregnancy positive population, showed a significant higher rates of ongoing pregnancy and live birth in BRM group which indicates a better maintenance of pregnancy in the BRM group compared to the long group. Moride and colleagues reported that using BRM in women with previous failed ART attempts is associated with better quality retrieved oocytes and morphology of embryos.[12] In addition, Mendes and colleagues revealed that transient hyperprolactinemia was associated with a more mature oocytes, higher numbers of follicles and better in vitro fertilization outcomes.[13]

In this clinical trial we demonstrated that the miscarriage rate in the BRM group was lower than the long protocol group. Jinno and colleagues have not addressed the rate of miscarriage in their work. Despite the similarity of number of oocytes retrieved and quality of embryos, the decline in miscarriage might be because of better endometrial receptivity. The findings of our study confirm a good endometrial receptivity which is more likely to be through improved endometrial prolactin system. In a pilot study, Garzia and colleagues have reported that a defect in endometrial prolactin production could result in unexplained infertility and repeated miscarriages.

The mean age of patients in this study was 7 and 6 years younger compared to Jinno and colleagues[10]which might explain the better outcomes of our study. The mentioned studies had been conducted on IVF failure while the current study included other patients which were not IVF failures.

The results and comments of this work have some limitation since the studied population was 114 women based on feasibility. The project could not recruit more volunteers and hence the results should be interpreted with caution. The findings of this study seem to open a new path toward understanding the endometrial receptivity, since it has a critical role in declining miscarriage in group of patients on BRM. However, the mechanism by which the miscarriage rate declines is still in need of explanation.

CONCLUSION

We concluded that BRM might lead to higher ongoing pregnancy and live birth rates compared to long protocol in women undergoing ICSI cycles.

CONFLICT OF INTEREST

None declared.

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Corresponding Author:
Fatemeh Sarvi, MD
Address: Department of Infertility, Shariati Hospital, North Karegar St., Near Gisha Bridge, Tehran, Iran.
Fax: +98 21 88008810
Tel: +98 21 88008810
Cell Phone: +98 9125463230
E-mail: fsarvi@razi.tums.ac.ir

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