Original Article

Value of Cabergoline and Low Dose Aspirin in Poor Responders Undergoing ICSI-ET Using Microdose GNRH Agonist Flare-Up-Protocol

Ayman Hany[1], Khaled M. Abdelaziz[2], Sherif El Sharkawy[1] and Radwa Fahmy[1]

Department of Obstetrics and Gynecology, Faculty of Medicine, [1]Cairo University, [2]Misr University for Science and Technology, Egypt.

ABSTRACT

Introduction: The aim of this study was to investigate pregnancy outcomes and live birth rate of vaginal progesterone gel protocol administered alone and and vaginal progesterone gel together with oral dydrogesterone protocol to provide luteal phase support in women undergoing *vitro* fertilization-intracytoplasmic sperm injection (IVF-ICSI).

Materials and Methods: This prospective, randomized, single blinded study was done in Kasr EI-Aini fertility and infertility center of Cairo University. The aim of the study was to determine the effect of cabergoline and low dose aspirin in poor responders undergoing ICSI-ET using microdose GnRH flare up protocol. The study involved 60 women referred to the center with the history of one or more failed IVF cycles with three or less retrieved oocytes. All women were assessed prior to inclusion with careful history taking, general examination and abdominal examination, and hormonal profile. Transvaginal ultrasound (TVUS) examination was done to assess antral follicle count (AFC). The 60 participants were allocated randomly into one of three treatment groups: CAB group (n=20) received cabergoline in a dose of 1 mg/week in two divided doses and low dose aspirin 80 mg/day, Aspirin group (n=20) received only aspirin as in CAB group and GnRH Group (n=20) received the microdose GnRH protocol only without any additive drugs.Ovarian stimulation was done using the Microdose flare-up regimen starting with the GnRH agonist leuprolide acetate 40 µg subcutaneously followed by stimulation with intramuscular injections of HMG in a daily dose of 300 IU/day. When at least 2 follicles 18 mm were observed on follicular monitoring, 10000 IU HCG were injected intramuscularly. Oocytes were retrieved 36 hours after hCG injection. After fertilization was confirmed, one to three grade A embryos were transferred at day 3 fertilization. Luteal phase support was then initiated from the day of oocyte retrieval for all patients. The primary outcome measure was the number of retrieved oocytes. The secondary outcome measures were number of fertilized oocytes, number of embryos transferred and ongoing pregnancy rate.

Results: The three groups were comparable in age (p = 0.509), body mass index (p = 0.221) and duration of infertility (p = 0.889). There was no significant difference between the three groups in the levels of FSH, LH and ANH (p = 0.808, 0.198, and 0.867). There was no significant difference between the three groups in the number of retrieved and fertilized oocytes (p = 0.852 and 0.990, respectively). Ongoing pregnancy was detected in 2 women (10%) of CAB group, 3 women (15%) of Aspirin group and 2 women (10%) in GnRH group with no significant difference between the three groups (p = 1.000).

Conclusions: In conclusion, the use of GnRH flare-up protocol in patients with poor ovarian response undergoing ICSI cycles achieved a pregnancy rate of 15%. The addition of low-dose aspirin to this protocol did not improve pregnancy rate in these cases. The triple therapy with cabergoline, aspirin and microdose GnRH was not effective in poor ovarian responders.

Key Words: Cabergoline, gnRH flare-up protocol, infertility, low-dose aspirin, patients with poor ovarian response.

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Corresponding Author: Ayman Hany Ahmed, MD, Department of Obstetrics and Gynecology, Faculty of Medicine, Cairo University, Egypt, **Tel.:** 010 0195 1615, **E-mail:** aymano_99@cu.edu.eg

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INTRODUCTION

Controlled ovarian hyperstimulation (COH) has contributed to the success of assisted reproduction techniques (ART), in *vitro* fertilization (IVF) and embryo transfer (ET). The efficacy of these techniques seems to depend on a personalized protocol of COH for each patient. The response to ovarian stimulation protocols is not always as expected or the same in many patients. Failure to respond to standard protocols and to recruit adequate follicles is called 'poor ovarian response'. This results in decreased oocyte production, cycle cancellation and, overall, it is associated with a significantly diminished probability of pregnancy[1].

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The etiology of poor response to ovarian stimulation is unknown. Despite being highly correlated with maternal age, the condition is also common in younger women in whom low ovarian reserve represents the most frequent etiological factor[2]. In addition, low ovarian reserve may be associated with advanced endometriosis, prior ovarian surgery, pelvic adhesions, increased body mass index, or smoking[3].

Although many protocols with different doses and types of gonadotropins have been proposed in the literature over the past 20 years for the management of poor responder patients, to date there is no really efficient treatment that could solve the problem of poor ovarian response. Although delayed start GnRH antagonist and microdose GnRH agonist were the two superior regimens in the treatment of poor ovarian response, providing favorable clinical outcomes[4].

However, although with the short flare-up protocols significantly more oocytes can be retrieved compared with GnRH antagonist protocols; the number of the retrieved oocytes is still significantly lower when compared with long standard GnRH regimens[5].

Supplementation with low-dose aspirin has been reported to have beneficial effects in selected groups of patients undergoing ART. It has been shown to be effective in enhancing uterine vascularity and pregnancy outcome in patients with impaired uterine blood flow undergoing thawed ET[6], as well as improving endometrial receptivity in oocyte donation recipients with a thin endometrium[7].

Cabergoline is used for the treatment of diseases caused by hyperprolactinemia and few neurological disorders (Parkinson's disease) as dopamine agonist. However, review of recent research suggests its possible application as a lipid lowering and anti-obesity agent by controlling serum prolactin levels. In a study on golden hamsters, it was observed that reduced prolactin retards liver lipogenesis even if insulin is available in abundance for supplying glucose for lipogenesis[8].

It has been found that cabergoline has a role in controlling the ovarian microenvironment through affecting the levels of growth factors (anti-Mullerian hormone, hepatocyte growth factor, insulin-like growth factor-1 and inhibin B) for folliculogenesis[9]. The aim of this study was to determine the effect of cabergoline and low dose aspirin in poor responders undergoing ICSI-ET using microdose GhRH agonist flare-up protocol.

PATIENTS AND METHODS

This study was designed as a prospective, randomized, single blinded study, from May 2015 to August 2018. It was conducted at Kasr EI-Aini fertility and infertility center of Cairo University. Sixty participants were assigned into the study, who were referred to the center with the diagnosis of poor ovarian response to hyperstimulation. All participants were prepared to undergo an ICSI trial after a failed previous cycle or more.

A signed informed consent was obtained from all the participants after explaining the purpose of the study. All participants were subjected to ovarian stimulation for ICSI cycles using microdose GnRH agonist flare up protocol.

Inclusion criteria

1. Patients with a history of one or more failed IVF cycles with three or less retrieved oocytes
2. No age limitation
3. Patients with an inadequate ovarian response in previous cycle
4. Low estradiol (E2) levels in response to ovarian stimulation in previous cycle
5. Lower number of retrieved oocytes in previous trials.

Exclusion criteria

1. Severe male factor (azospermia)
2. Hydrosalpinx
3. History of endometriosis
4. Endocrine or metabolic disorders
5. Follicle stimulating hormone (FSH) level > 15 mIU.
6. Antimullerian hormone (AMH)< 0.5 ng/ml
7. Any uterine causes of infertility e.g. septate uterus

The 60 participants were divided by random allocation computer program into three groups: (all are stimulated with microdose GnRh flare up protocol.

1. CAB group: (n=20) received cabergoline in addition to aspirin. Cabergoline was administered in a dose of 1 mg/week in two divided doses which was terminated after embryo transfer. Aspirin was administered in daily dose of 80 mg initiated at the start of down-regulation with luteal leuprolide.
2. Aspirin group: (n=20) received only aspirin as in CAB group.
3. GnRH Group: (n=20) the microdose GnRH protocol only without any additive drugs.

Assessment of Participants

Each patient was subject to the following

1. Full history taking
2. Full clinical examination including: General examination and Abdominal examination
3. transvaginal ultrasound (TVUS) examination on day 2 of the cycle to assess antral follicle count (AFC)
4. Office Hysteroscopy
5. Laboratory instigations including:
    a. FSH
    b. LH
    c. AMH
    d. E2
    e. TSH

Ovarian stimulation was done using the Microdose flare-up regimen. The patient received diluted doses of the GnRH agonist leuprolide acetate 40 µg, given subcutaneously twice daily. Two days later, stimulation is initiated by intramuscular (IM) injections of HMG (Merional, IBSA, Germany) in a dose of 300 IU/day. Follicular monitoring began from the ninth day of the cycle by serial vaginal ultrasonography and measurement of serum E2 levels. IM injections of 10000 IU HCG (Choriomon; IBSA, Germany) were injected when at least 2 follicles 18 mm were observed on ultrasonography.

Oocytes were retrieved 36 hours after hCG injection using a 17-gauge aspiration needle under transvaginal ultrasound guidance. The pelvis is evaluated with ultrasound to ensure that there is no internal bleeding.

After fertilization was confirmed when two polar bodies and two pronuclear were observed 18-20 hours after insemination, one to three grade A embryos were transferred at day 3 fertilization.

The luteal phase support was initiated from the day of oocyte retrieval for all patients with (Cyclogest 400 mg, Actavis pharmaceutical, UK) vaginal suppositories per day till the day of serum pregnancy test was done.

Serum β-hCG was performed 14 days after the embryo transfer to confirm chemical pregnancy. Clinical pregnancy was confirmed when there was an evidence of gestational sac, embryo and fetal heart activity at time of transvaginal ultrasound evaluation by the 6th week.

Outcome measures:

The primary outcome measure was the number of retrieved oocytes.

Secondary outcome measures were

1. Number of fertilized oocytes
2. Number of embryos transferred
3. Ongoing pregnancy rate (defined as viable pregnancy when there is evidence of gestational sac, embryo and fetal heart activity at time of transvaginal ultrasound evaluation by the 6th week)

Statistical Methods

Statistical analysis was done using IBM© SPSS© Statistics version 22 (IBM© Corp., Armonk, NY, USA). Numerical data were expressed as mean and standard deviation or median and range as appropriate. Qualitative data were expressed as frequency and percentage. Chi-square test (Fisher's exact test) was used to examine the relation between qualitative variables. For quantitative data, comparison between the 3 groups was done using Kruskal-Wallis test followed by the appropriate post-Hoc test. A *p-value* < 0.05 was considered significant.

RESULTS

The study involved 60 women subjected to controlled ovarian stimulation for ICSI cycles using microdose GnRH agonist flare up protocol. They were divided into 3 groups. CAB group received cabergoline in addition to aspirin, while Aspirin group received only aspirin. The third group received the microdose GnRH protocol only without any additive drugs. The three groups were comparable in age (p = 0.509), body mass index (p = 0.221) and duration of infertility (p = 0.889) as shown in (Table 1, Figures 1, 2).

Table 1: Baseline characteristics of the three studied groups

	CAB Group n=20	Aspirin Group n=20	GnRH Group n=20	*p value*
Age (years)	33.8±3.9	33.7±3.4	32.5±3.9	0.509
BMI (kg/m2)	27.3±1.3	28.1±2.5	26.9±2.4	0.221
Duration of infertility (years)	4.4±1.6	4.7±2.1	4.5±2.2	0.889

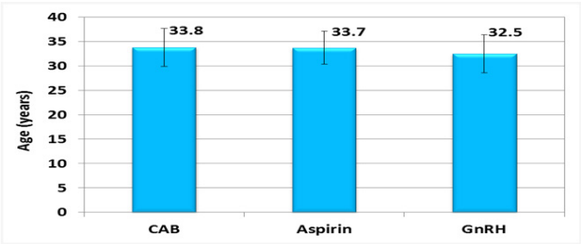

Fig. 1: Mean age of the three studied groups

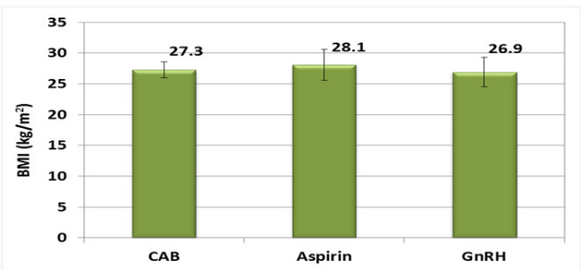

Fig. 2: Mean body mass index of the three studied groups

(Figure 3) shows that the majority of patients in the three groups were suffering primary infertility with no significant difference between groups (p = 0.926). (Table 2) shows that some of the males in the treated couples had sperm abnormalities in semen analysis. However, the proportion of abnormal semen analysis was not significantly different between the three groups (p = 0.116).There was no significant difference between the three groups in the levels of FSH, LH and AMH (Table 3). There was no significant difference between the three groups in the number of retrieved and fertilized oocytes (Table 4). As shown in (Tables 5,6). There was niether significant difference between the three groups regarding the number of days of merional use (P = 0.125) nor the total dose of merional (p = 0.056).

Table 2: Results of semen analysis of the husbands of the three studied groups

	CAB Group n=20	Aspirin Group n=20	GnRH Group n=20
Normal	17 (85.0%)	13 (65.0%)	11 (55.0%)
Asthenospermia	2 (10.0%)	3 (15.0%)	5 (25.0%)
Oligospermia	1 (5.0%)	2 (10.0%)	2 (10.0%)
Oligoasthenospermia	0 (0.0%)	2 (10.0%)	2 (10.0%)

Table 3: Hormone profile of the three studied groups

	CAB Group n=20	Aspirin Group n=20	GnRH Group n=20	*p value*
FSH (mIU/ml)	10.1±1.8	9.9±1.8	9.7±1.8	0.808
LH (mIU/ml)	4.8±1.1	4.6±1.7	4.0±1.3	0.198
AMH (ng/ml)	0.70±0.33	0.69±0.33	0.75±0.35	0.867

Table 4: Number of retrieved and fertilized oocytes in the three studied groups

	CAB Group n=20	Aspirin Group n=20	GnRH Group n=20	*p value*
No. of retrieved oocytes	2 (0-3)	2 (0-4)	2 (0-4)	0.852
No. of fertilized oocytes	2 (0-3)	2 (0-3)	1 (0-3)	0.990

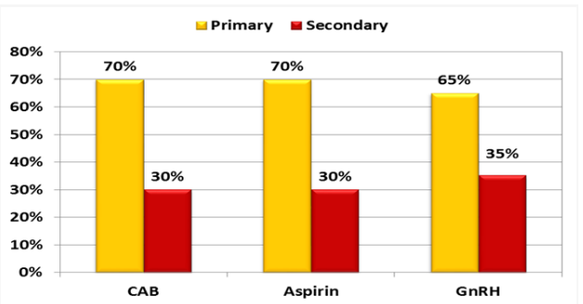

Fig. 3: Type of infertility in the three studied groups

The ICSI cycle resulted in few pregnancies; 2 in CAB group, and 3 in both Aspirin and GnRH groups (Table 7). There was no significant difference between the three groups in the ongoing pregnancy rate (p = 1.000) (Figure 4).

Table 5: Number of days of merional intake in the three studied groups

	CAB Group n=20	Aspirin Group n=20	GnRH Group n=20
Number of days			
Median (Range)	14 (13-15)	14 (13-15)	14 (12-15)
p value		0.125	

Table 6: Total dose merional in the three studied groups

	CAB Group n=20	Aspirin Group n=20	GnRH Group n=20
Dose of merional (IU)	4155±427	4140±431	3840±408

Table 7: Outcome of ICSI cycle in the three studied groups

	CAB Group n=20	Aspirin Group n=20	GnRH Group n=20
Positive pregnancy	2 (10%)	3 (15%)	3 (15%)
Negative pregnancy	18 (90%)	17 (85%)	17 (85%)

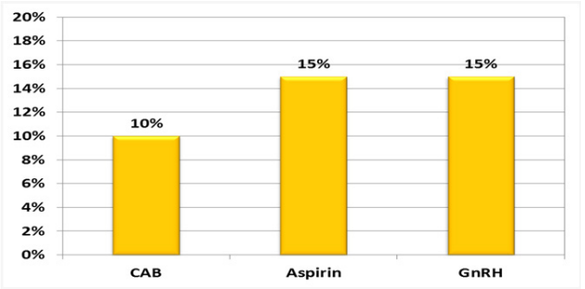

Fig. 4: Outcome of ICSI cycle in the three studied groups

DISCUSSION

In the current study, we used micro dose GnRH agonist micro dose flare protocol for the management of 60 women responding poorly to controlled ovarian hyperstimulation in previous ICSI cycles. In a randomized clinical trial including 120 women with histories of one or more failed IVF cycles, microdose flare-up regimen resulted in a pregnancy rate of 10%[10]. Another study reported higher rate of 26%[11] concluded that the flare-up protocol is more effective than the GnRH-antagonist protocol in terms of mature oocytes retrieved, fertilization rate, and top-quality embryos transferred in poor-responder patients. It was also reported that the clinical pregnancy rate after GnRH agonist microdose flare-up protocol was similar in comparison to GnRH antagonist protocol in poor ovarian responders[12]. And we tried to enhance the results of the treatment cycles by adding low dose aspirin only to one group (n=20) and aspirin combined with cabergoline to another group (n=20).

The rationale of using low-dose aspirin in poor ovarian responders in the current study was based on its vasoactive effect that can enhance ovarian vascularization[6]. Ovarian vascularity and folliculogenesis have been reported to be closely related in both natural and stimulated cycles. Increased intraovarian vascularity has been linked to preferential delivery of gonadotropic hormones or other growth factors required for folliculogenesis[13]. Impaired ovarian blood flow with high resistance appears to be a contributing factor to poor ovarian response[14].

In our current study, ongoing pregnancy rate was not enhanced in patients receiving low-dose aspirin in comparison to those on GnRH agonist only (p = 1.000).In agreement with the current study, a meta-analysis including 10 studies concluded that aspirin does not improve pregnancy rates after IVF[15]. Also, in agreement with the current study, in poor responders to ovarian stimulation, previous investigators did not find aspirin to be effective in improving pregnancy rate[16].

It was reported that a transient rise in plasma prolactin (PRL) concentrations can be observed during the late follicular and luteal phases of both natural and stimulated cycles[17]. It is known that hypothalamic dopamine is the major inhibitor of PRL secretion in humans and there may be a possible, if controversial, role for central dopaminergic mechanisms in the release of LH[18]. Several investigators also indicated a dopaminergic control on gonadotrophin secretion, and suggested that a reduction of the dopamine inhibitory effect might cause abnormal PRL and LH release, as found in hyper PRL–PCOS patients[19].

It has been found that cabergoline has a role in controlling the ovarian microenvironment through affecting the levels of growth factors (anti-Mullerian hormone, hepatocyte growth factor, insulin-like growth factor-1 and inhibin B) for folliculogenesis[20].

Cabergoline lowers serum prolactin levels by acting on dopaminergic (D2) receptors in central nervous system (CNS). Also, it has been reported that the body has a homeostatic mechanism for controlling body fat and weight through CNS. Flier had summarized many possible pathophysiological mechanisms involved in the development and maintenance of body fat and weight[21].

CONCLUSION

The results of the current study did not demonstrate an additive effect of cabergoline when combined to low dose aspirin in improving ongoing pregnancy rate. Chang *et al*. reached the same results. He evaluated the correlation between prolactin and estradiol plasma levels 36 hours before, 12 hours before and 12 hours after hCG administration at mid-cycle, and confirmed that no treatment was needed in IVF patients with transient hyperprolactinemia. The role of prolactin on responsiveness of granulosa cells in oocyte maturation is still unknown, even if in the last few years, the literature supports a positive effect of transient hyperprolactinemia. Our data confirm the hypothesis that a transient increased level of prolactin improves oocyte quality and fertilization rate, and that not treating transient hyperprolactinemia is the best approach, in order to increase IVF program success.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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