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Research Article

A Prospective Comparative Interventional Study of Dydrogesterone (Progestin Primed Ovarian Stimulation) Versus Ganirelix Acetate (GnRh Anatgonist) for Freeze-all IVF/ICSI Cycles

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Abstract

Background: With advancement in IVF technology and vitrification, use of progesterone and its derivatives to block the LH surge is increasing. This was a prospective randomized controlled trial, in a private reproductive medicine clinic to compare the efficacy of preventing LH surges by using progesterone primed ovarian stimulation (PPOS) against gonadotropin-releasing hormone (GnRH) antagonist, in women with good ovarian reserve undergoing *in vitro* fertilization (IVF).

Method/Design: Study participants (n = 220) who met the inclusion criteria were randomized. Both group participants received hMG from day 2. Group 1 (hMG + DYG) received 10mg tablet Dydrogesterone, thrice a day, from day 2 of stimulation. Group 2 (hMG + GAN) received 0.25 mg of Ganirelix acetate, given subcutaneously from fifth day of stimulation till the day of trigger. Main Outcome Measure(s): The primary outcome measured was the incidence of premature LH surges. All embryos were frozen and transferred in the subsequent cycle. Participants were monitored by serial hormonal assays.

Main Outcome Measure (s): The primary point of measurement was the incidence of premature LH surges. We also studied the number of oocytes retrieved, number of embryos formed, implantation and clinical pregnancy rate along with the additional cost per cycle as our secondary end parameters.

Result (s): All subjects in both groups had well controlled LH levels suggesting good control over prevention of premature leutinisation. There was no significant difference in the number (mean ± SD) of oocytes retrieved [P = 0.0691] and viable embryos or the pregnancy rate (PR) after FET 48% {43.64, median (IQR)} versus 39% {(35.45, median (IQR)} (P = 0.215). The additional cost per cycle was significantly high in the antagonist group (p < 0.001).

Conclusion: Our results suggest that Dydrogesterone effectively inhibited spontaneous ovulation, without affecting the number of retrieved oocytes, embryo quality, implantation and pregnancy rates (PR). The cost difference and patient acceptance between both the groups was significant with Dydrogesterone allowing lower costs and easier (oral) administration thus making IVF cycle more patient friendly.

Keywords: LH Surge; Dydrogesterone; Ganirelix Acetate; GnRh Antagonist; PPOS; Freeze All

Introduction

Ovarian stimulation is employed aiming to stimulate the growth of several follicles and, consequently, to obtain as many high-quality oocytes as possible [1]. In the routine conventional ovarian stimulation protocols, the serum FSH and LH levels are maintained above the crucial level with the use of exogenous gonadotrophins. This helps in the growth of many follicles at a time [2]. The early rise in oestradiol concentrations, due to the development of multiple follicles at the same time, may promote an extemporaneous LH surge, leading to spontaneous ovulation. We have been using GnRH and its analogues to maintain pituitary suppression and avoid spontaneous ovulation. In GnRh antagonist cycles, final maturation of the oocyte and ovulation are triggered using a bolus of GnRH agonist, human chorionic gonadotrophin (HCG; a hormone that is biologically similar to LH but with a longer half-life) or both [1].

GnRH antagonists promptly suppress pituitary gonadotrophin by GnRH-receptor competition [2]. Once the antagonist is given, gonadotrophin secretion decreases within hours and hence there is no flare-up. Once the GnRH antagonist is stopped, there is a quick and predictable recovery of the hypothalomo-pituitary-gonadal axis as the pituitary receptor system is intact [3]. This protocol has fewer complications and are convenient for patients because of the shorter treatment time and lower number of injections as compaered to the long agonist protocol. However, according to studies comparing a GnRH agonist and GnRH antagonist protocol, the number of oocytes and embryos obtained are significantly lower in antagonist cycles [4]. A varied proportion (0.34-38%) of patients using a GnRH antagonist has been demonstrated to experience a premature LH surge, especially seen in older women and women with diminished ovarian reserve [5]. Also this protocol increased the cost to the patient. Due to these drawbacks, fertility specialists are analyzing and researching various other options to avoid premature LH surges.

The major regulatory factors of the gonadotrophin surge have been identified as hypothalamic GnRH, ovarian steroids such as oestradiol and progesterone, and various other regulatory factors such as cytokines, leukotrienes and glucocorticoid, adrenergic and dopaminergic stimuli. The crucial commanding element which regulates gonadotrophin concentrations is ovarian steroid effect on the anterior pituitary. As the estrogen concentration increases with increase in size of growing follicles, a critical level is reached which triggers the final maturation of the oocytes and making it ready for ovulation. This leads to a positive feedback on LH production and finally the LH surge. This LH surge increases intrafollicular proteolytic enzymes, weakening the wall of the ovary and allowing the mature follicle to pass through [6]. This change from a negative to positive feedback on LH secretion happens via both the pituitary gland and the hypothalamus. There is an increased response to GnRH as there is increase in their receptors present on the gonadotrophic cells. This could be due to an increase in the GnRH of pituitary gland because of hindrance of GnRH metabolism, leading to LH secretion [7,8].

We are still unaware about the neural mechanisms by which progesterone and oestradiol interact and regulate the gonadotrophin surge. The actions of progesterone action may be synergistic with, or antagonistic to, the actions of oestradiol, depending on hormone ratios and the timing of exposure [9]. Progesterone seems to have a permissive role in the preovulatory LH peak: experiments have shown a rise in progesterone preceding the LH surge by several hours in the pre-ovulatory period [10]. In various studies, exogenous progesterone has been shown to induce a peak in LH if administered to oestrogen-primed women [11]. We also know that progesterone has a negative effect on ovulation. Various studies on use of progesterone for contraception has shown that it blocks the LH surge [12,13]. The progestin-only contraceptive pills (POP) use this inhibitory action of progesterone on growth of follicle and ovulation as abasis for their mechanism of action for contraception.

Changes in progesterone cause dramatic modifications in GnRH pulse frequency: its removal induces an acceleration of the pulse generator, while its administration slows the pulse frequency, with LH secretion being consequently modified [14]. Progesterone priming slows LH pulse frequency and reduces the mean plasma LH concentrations [15].

With advancement in cryopreservation techniques, we can now maintain the quality of embryo and oocytes. This has given us the liberty to move away from the standard protocol of ovarian stimulation-retrieval-transfer. Today many centers are following the 'freeze-all' strategies to prevent ovarian hyperstimulation (OHSS) in women or to avoid the determinantal effects of high estrogen on the endometrial lining which can adversely affect

the embryo implantation [16]. In 2015, Dr Yanping Kuang first suggested the use of progestin-primed ovarian stimulation (PPOS), as a new ovarian stimulation regimen. He combined progesterone supplement with exogenous gonadotrophin, and used GnRH agonist along with hCG as the triggers. When progestin is administered in the early phase of the cycle, it can inhibit the pre-ovulatory LH surge before oestrogen priming can happen [17-19]. In order to circumvent a low response of the hypothalamic pituitaryovarian (HPO) axis, they gave dual trigger with GnRHa along with low dose of hCG (1000IU) and induced final oocyte maturation. Further due to the very low dose of HCG, the risk of moderate or severe OHSS was minimal [20,21].

When a natural exogenous progesterone like Utrogestan is used, it can hinder the measurement of serum progesterone measurement and lead to the delinquency in a possible premature luteinization. When Medroxy progesterone acetate (MPA) was used it lead to stronger pituitary suppression and therefore higher dosage of gonadotrophin requirement as well as longer duration of ovarian stimulation [20]. Hence the need to find a better synthetic progestins which would be most acceptable for PPOS. Dydrogesterone (DYG) has been used in early pregnancy and has been found to be safe during embryogenesis. Hence this molecule will probably not adversely affect the oocyte quality or fertilsation.

In this study we compared the effectiveness of Dydrogesterone (DYG) versus Ganirelix acetate (GAN) in prevention of premature LH surge and inhibit ovulation in controlled ovarian hyperstimulation (COH) cycles for women with normal to good ovarian reserve undergoing IVF/ ICSI treatment. Dydrogesterone was started from the day 2 of ovulation induction and found it to be equally effective in preveting premature LH surge without adversely affecting the quality of oocytes or the embryos.

Materials and Methods

This was a prospective, comparative, interventional study conducted in a private infertility center performed from October 2020 to September 2021. We included 220 women undergoing standardized COH protocols for IVF/ ICSI who were for "Freeze all cycle". Only women who fulfilled our inclusion criteria were thoroughly informed about the study and then included. Their complete history was taken, including any exposure to radiation or hazardous chemical substances, IV drug use and reproductive history. These women had normal gynecological and physical examination, with no family history of hereditary or chromosomal diseases, normal karyotype, and no sexually transmitted diseases (STDs). All women had to give a written and informed consent before enrollment. Local ethical committee approval was obtained.

The following women were included in the study:

- Women who have a history of infertility ≥1 year
- Women with regular menstrual cycles of 25- 35 days over the previous 3-month period
- Body mass index - 18 to 29
- USG pelvis - normal with presence of both ovaries
- AFC > 12 on day 2/3 of menstrual cycle
- AMH > 2.8
- Basal levels of oestradiol (≤ 50 pg/ml) on day 1 of stimulation
- Basal serum FSH concentration of no more than 10 IU/L.
- All patients had to sign an informed consent.

Exclusion criteria: Women who met any of the following criteria are excluded:

- Documented ovarian failure including basal FSH above 10 IU/L
- Presence of a functional ovarian cyst with E_2>100 pg/mL
- Receipt of hormone treatments within the previous 3-month period
- Endometriosis grade 3 or higher Premature ovarian insufficiency Any contraindications to ovarian stimulation treatments Unable to comply with the study procedures Clinically significant systemic diseases, such as renal failure and systemic lupus erythematosus Known Müllerian anomalies Abnormal vaginal bleeding of unknown etiology.

Randomisation

Participants were allocated randomly into one of the two arms on the 2nd day of menstrual cycle at a ratio of 1:1. They were allocated using computer-generated random numbers. Neither the investigators nor the participants were aware of the allocation after ovarian stimulation. The fertility specialist as well as the embryologists were blinded to the group issuance.

Protocols

PPOS protocol

Ovarain stimulation was started with 225 - 300 IU of human menopausal gonadotropin (hMG) administered daily from menstrual cycle day 2. Dydrogesterone (DYG) was started at a dose of 10mg thrice daily orally from the second day of stimulation and continued till day of trigger. The dosage of gonadotrophins was adjusted according to the ovarian response. The final stage of oocyte maturation was induced only when the leading follicles reached a diameter of 18 mm, with 0.2mg of triptorelin along with HCG 1000 IU. The oocytes were retrieved performed 34-36 hrs after the trigger. Tab Cabergoline 0.5 mg was started after trigger for eight days to prevent OHSS in both the groups.

GnRH antagonist protocol

COS was started in a similar fashion. From the fifth day of injection, when the dominant follicle reached a size of 13-14 mm, 0.25 mg ganirelix acetate (GAN) was administered daily up to the day of trigger (fixed protocol). Rest of the protocol remained same in both the groups.

All follicles which were more than 10 mm in size were retrieved transvaginally. The oocytes were fertilized *in vitro*, by IVF or ICSI, depending on semen count and motility. The number and regularity of the embryos were checked and documented, along with regularity of blastomeres and fragmentation in embryos on the third day [31]. All good-quality grade 1 and grade 2 cleavage-cell embryos were frozen on the third day after retrieval. The other lower grade embryos were cultured till they reached the blastocyst stage or disintegrated. The good grade blastocysts were frozen on the fifth or sixth day. The cleavage-stage embryos and blastocysts were frozen using the Cryotop carrier system (Kitazato Biopharma Co., Fuki, Japan) containing a mixture of 15% (v/v) ethylene glycol, 15% (v/v) dimethyl sulfoxide and the cryoprotectant was 0.5M sucrose. Solutions of 1, 0.5 and 0M sucrose were used sequentially as cryoprotectant dilutions during thawing. The first warming step was conducted at 37°C followed by vitrification and warming done at room temperature [31].

Endometrium preparation in subsequent cycles with hormone replacement therapy (HRT). Estradiol valerate was administered orally at 4 - 6mg/day from cycle day 2 onwards, roughly for 14 to 16 days. Once the endometrial lining was more than 8 mm thick, progesterone was added daily via an intravaginal route and orally also. We transferred blastocysts on P^{0+5} days, that is on the 6^{th} day of starting progesterone. We transferred either one or two embryos per cycle. Patients were followed up till their serum beta HCG values and the ones that had conceived were further followed up till first ultrasonography suggesting viable pregnancy. Dydrogesterone was given at a dose of 30 mg/ day orally while micronised progesterone (200 mg/ day) twice vaginally was used for luteal support.

Hormonal measurement

During the COH, various hormones were measured like serum follicle-stimulating hormone (FSH), LH, oestradiol and progesterone. These hormones were measured via chemiluminescence technique. A baseline serum level of all these hormones was done on day 1 of menstrual cycle. The serum FSH, LH, oestradiol and progesterone levels were serially repeated on day 6 of controlled ovarian stimulation (COS), on the day of trigger and 12 hours after trigger.

Outcome measurements

Primary outcome

The primary outcome measured was the incidence of premature LH surges. This was defined as a LH level of more than 15 mIU/ml in blood on the day of trigger. This did not require the rupture of dominant follicle or an increase in serum progesterone. If the dominant follicle ruptured before the scheduled time it was labelled as premature ovulation. LH surge did not mean just an increase in progesterone alone and was hence noted separately.

Secondary outcomes

The duration and dosage of gonadotrophins used for COH, serum hormone measurements, number of oocytes obtained along with mature oocytes, fertilization rate, viable embryos per oocyte retrieved, cycle cancellation rate and pregnancy outcomes from the first FET cycle were the secondary outcomes studied. The viable embryos meant grade 1 and grade 2 cleavage cell embryos on day 3 and good-grade blastocysts on Day 5 or 6 of development obtained from non-top-quality embryos after 3 days of oocyte retrieval. When there were no viable embryos that were frozen for later transfer it was considered as cycle cancellation. Pregnancy

was defined by a positive beta HCG value of more than 100 IU done on day 14 post embryo transfer. The ultrasound finding of a gestational sac with or without fetal heart activity, 4 weeks after embryo transfer was defined as clinical pregnancy. The number of gestational sacs divided by the number of embryos transferred was designated as implantation rate.

Randomization

The participants were randomized at the OPD, once we got the results of the baseline hormonal analyses after discussion with the participants. Women were randomized using a computer-generated list. Each patient was enrolled only once in the study. The OPD doctors were not blinded from the study. The fertility specialist doing the oocyte retrieval, embryologists and statisticians were blinded to group assignments.

Data management

Table 1 outlines the juncture of enrolment, intervention, data collection and follow-up of the participants.

Timepoint	Study period					
	Enrollment	Baseline appt	Allocation	Post allocation		
		No more than 4 weeks	Menstrual cycle day 1	Cycle monitor	Oocyte retrieval	FET
Enrollment						
Identification	X					
Eligibility screening	X					
Completion of baseline measures		X				
Informed consent		X				
Randomisation			X			
Allocation			X			
Interventions						
PPOS protocol				X	X	
GnRh antagonist protocol				X	X	
Data Collection						
Demographics		X				
Primary outcome measure				X		
Secondary outcome measure					X	X
Treatment attendance						
Adverse effects						

Table 1: SPIRIT diagram for this protocol comparing PPOS with GnRH antagonist in IVF/ICSI cycles.

Statistics

This study included 220 participants, with 110 in each group. Statistical treatment was performed with SPSS 7.0 software. Chi-square test was used to examine differences in the incidence of premature LH surges. The comparison of serum hormones at different time stages within each group was done using covariance analysis. Pearson's chi square test or Fisher's exact test was used to assess count data which were presented as numbers and percentages. Logistic regression was used to calculate the odds ratio (OR) and 95% CI. For comparing baseline data between the two groups, two-sided $P < 0.05$ was considered statistically significant. While for trial contains a multitude of secondary endpoint, two-sided $P < 0.01$ was considered significant. Chi-square test was used for enumeration data of the secondary efficacy parameters while

measurement data was tested using Student's t test. P value of less than 0.05 was considered statistically significant.

Observation and Results

Patient characteristics

Table 2 outlines the baseline characteristics of the patients in both COH stimulation groups. There were no differences in age, BMI, AMH, Antral follicular count (AFC), infertility duration, type and cause of infertility between the two groups as seen in table 2 ($p > 0.05$).

Items	Group 1 (n =110) Dydrogesterone 30mg	Group 2 (n = 110) Ganirelix 0.25 mg s/c	P value
	Median (IQR)	Median (IQR)	
Age (years)*	25.84 (2.91)	26.05 (2.67)	0.5795
BMI (kg/m^2) *	21.3+/-2.5	22.3+/-1.5	0.68
AMH*	6.2+/-0.4	5.8+/-0.5	0.98
AFC*	20+/-5.5	19+/-6.2	0.96
Infertility duration (years) *	2+/- 0.5	1.9+/-0.5	1.2
Primary infertility n (%)	110 (100)	110 (100)	-----
Indication, n (%)			
Tubal	48 (44)	44 (40)	-----
Male factor	18 (16)	22 (20)	-----
Unknown factor	7 (06)	11 (10)	-----
Combined factor	37 (34)	33 (30)	-----

Table 2: General data in the Dydrogesterone (Group 1) and Ganirelix group (Group 2).

BMI- Body mass index, AMH- Anti mullerian hormone, AFC- Antral Follicular count.

Values are presented as Median (IQR) and p value is obtained from Mann whitny test.

(*)- Values are presented as Mean (SD) and p value is obtained from Independent t-test.

($)- Values are presented as number (%) and p value is obtained from Chi-square test.

Baseline hormonal profile

Baseline hormonal profile was tested on day 1 of menses, before stating the controlled ovarian hyperstimulation (COH). There were no differences in serum FSH, LH, Estradiol and progesterone values on day 1 of the menstrual cycle ($p > 0.05$) as seen in table 3.

Items	Group 1 (n =110) DYG 30mg	Group 2 (n = 110) GAN 0.25 mg s/c	P value
	Median (IQR)	Median (IQR)	
Day 1 Sr. FSH*	3.56 (2.3,4.5)	3.5 (2.1,3.5)	0.4573
Day 1 Sr. LH*	1.6 (1.98,4.6)	1.8 (1.5,2.4)	0.1355
Day 1 Sr. Estradiol (E2) *	32.4 (10.21)	32.9 (10.34)	0.3576
Day 1 Sr. Proges-terone*	0.20 (0.1,0.3)	0.23 (0.2,0.4)	0.7641

Table 3: Baseline honormones in the Dydrogesterone (group 1) and Ganirelix (group 2).

Values are presented as Median (IQR) and p value is obtained from Mann whitny test.

(*)- Values are presented as Mean (SD) and p value is obtained from Independent t-test.

Hormone profile during treatment

The hormonal levels of serum FSH, LH, E2 and progesterone were monitored throughout the stimulation and repeated 12 hours post trigger in both the group as seen in table 4.

Hormone	Day of stimulation	Group 1 (n =110) DYG 30mg	Group 2 (n = 110) GAN 0.25 mg s/c	P value
Sr. FSH (IU/L)*	Baseline	3.56 (2.3,4.5)	3.5 (2.1,3.5)	0.4573
	Day 6	14+/-4.5	13.8+/-4.8	0.194
	Day of trigger	17.86+/-5.2	17.9+/-5.4	0.256
Sr Estradiol (E2) (IU/L)*	Baseline	32.4 (10.21)	32.9 (10.34)	0.3576
	Day 6	792 (559,1155)	895 (575,1123)	0.5566
	Day of trigger	2896 (2485,3256)	2787 (2552,3215)	0.8314
Sr. Progesterone (IU/L)*	Baseline	0.20 (0.1,0.3)	0.23 (0.2,0.4)	0.7641
	Day 6	0.21 (0.1,0.3)	0.23 (0.1,0.5)	0.4321
	Day of trigger	0.98 (0.5,1.1)	0.9 (0.6,1.2)	0.1584
Sr. LH (IU/L)*	Baseline	1.6 (1.98,4.6)	1.8 (1.5,2.4)	0.1355
	Day 6	1.9(1.2,3.5)	1.64(1.3,2.4)	0.1643
	Day of trigger	1.2 (0.32,1.7)	1.28 (1.1,1.9)	0.8219
Sr. LH (IU/L)*	12 hours after trigger	27.92 (6.7)	27.83 (5.89)	0.9871

Table 4: Hormone levels during the course of COH.

Values are presented as Median (IQR) and p value is obtained from Mann whitny test.

(*)- Values are presented as Mean (SD) and p value is obtained from Independent t-test

The serum FSH increased steadily and significantly during COH in all the study particpiants. An elevation of serum LH level above 10 IU/L and a serum progesterone level of more than 1ng/mL is commonly defined as premature LH surge (European and Middle East Orgalutran Study, 2001; Lambalk., *et al*. 2006). In this study the serum LH level was less than 10 IU/L in all participants showing that progestins could effectively subdue a premature LH surge. Serum LH levels increased significantly 12 hrs after trigger ($P < 0.001$). In both the groups, the serum estradiol levels gradually rose along with the growth of follicles during COH. However, there was no difference between the groups in this respect. The serum progesterone was at a low level in both the groups during the COH.

Ovarian stimulation, follicle development and ocyte performance

Table 5 summarizes the cycle characteristics, ovarian stimulation and embryological data in both groups. The total hMG dose requirement {2500(2475,2750) vs 2500(2250,2750); p = 0.3691} and the duration of stimulation {10.09(0.49) vs 10.04(0.59), p = 0.5403} was similar in both groups. The mean number of oocytes retrieved was 26 (24,33) in group 1, and 25 (22,29) in group 2, (P = 0.0691). The number of fertilized oocytes {21(19,29) vs 20(18,24), p = 0.0467) and embryos frozen on day 3 {20(18,27) vs 20(18,24), p = 0.9525} was also similar in both groups. During this study, none of the subjects suffered from moderate or severe OHSS, neither were any cycles cancelled.

Characteristics	Group 1 (n =110) DYG 30mg	Group 2 (n = 110) GAN 0.25 mg s/c	p value
	Median(IQR)	Median(IQR)	
HMG requirement*	2500(2475,2750)	2500(2250,2750)	0.3691
Days of stimulation *	10.09(0.49)	10.04(0.59)	0.5403
Oocytes retrieved	26(24,33)	25(22,29)	0.0691
M II	21(20,29)	22(20,25)	0.4469
M1,GV	4(3,5)	3(2,4)	0.0004
Fertilised oocytes	21(19,29)	20(18,24)	0.0467
Embryos frozen on Day 3	20(18,27)	20(18,24)	0.9525
Oocyte retrieval rate (%)	74.07+/-18.34	71.42+/-20.34	0.69
Incidence of mod and severe OHSS (%)	0	0	------

Table 5: The cycle characteristics of controlled ovarian stimulation in two groups.

Note: Values are presented as Median (IQR) and p value is obtained from Mann whitny test.

(*)- Values are presented as Mean (SD) and p value is obtained from Independent t-test.

Pregnancy outcomes in FET cycles

During the 5 months of follow-up, all recruited study participants had one cycle of embryo transfer. The pregnancy rate was comparable in both groups as seen in table 6.

Characteristics	Group 1 (n =110) DYG 30mg	Group 2 (n = 110) GAN 0.25 mg	P value
Thawed embryos (n)	180	178	0.306
Viable embryos after thawing (n)	162	161	0.392
Transferred embryos (n)	1.7+/-0.2	1.8+/-0.3	0.240
Endometrial preparation (n)	110	110	---
Endometrial thickness (mm)	11.6+/-2.4	11.2+/-2.2	0.440
Pregnancy outcome of FET (%)			
Biochemical pregnancy rate per transfer n(%) $			0.215
Positive	48(43.64)	39(35.45)	
Negative	62(56.36)	71(64.55)	
Clincal pregnancy rate per transfer	45.45% (50/110)	37.27% (41/110)	0.07
Implantation rate	30.86% (50/162)	25.46% (41/161)	0.854

Table 6: Pregnancy outcome of frozen thawed embryos originating in the two groups.

Values are presented as Median (IQR) and p value is obtained from Mann whitny test.

(*)- Values are presented as Mean (SD) and p value is obtained from Independent t-test.

($)- Values are presented as number (%) and p value is obtained from Chi-square test.

The additional cost per IVF/ICSI cycle in Dydrogesterone group was 32 USD as compared to 177 USD ($p < 0.001$). The patient satisfaction was studied using the 'Short Assessment of Patient Satisfaction (SAPS) questionnaire' as seen in figure 1.

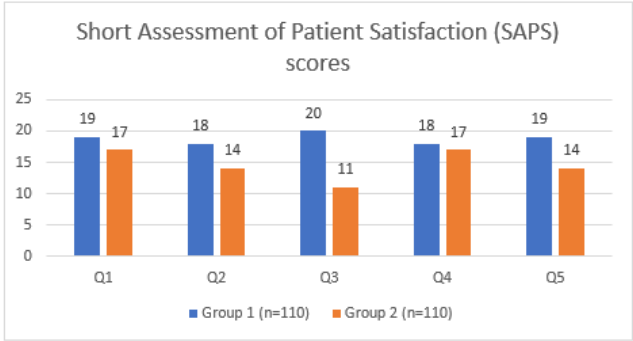

Figure 1: Short Assessment of Patient Satisfaction (SAPS) scores in (group 1) PPOS and (group 2) GnRH antagonist groups.

Q1; How satisfied are you with the explanations the doctor has given you about the your treatment?, Q2; Are you satisfied with the route of drugs (oral vs. injectable) being given to you?, Q3; Are you satisfied with the ease of taking these drugs (oral vs. injectable) being given to you?, Q4; Are you satisfied with the care you received in the clinic?, Q5; Are you satisfied with the overall treatment?, Scores: 1 to 5; Very dissatisfied, 5 to 10; Dissatisfied, 11 to 15; Satisfied, and 15 to 20; Very satisfied.

Results

These findings of this study propose the possible use of Dydrogesterone as a better progestin for PPOS protocol in IVF/ICSI. Progestin-primed ovarian stimulation (PPOS) can be considered as the first option for COH in women undergoing fertility preservation, oocyte donation or preimplantation genetic testing. Other protocols wherein oocyte or embryo cryopreservation is essentially needed, like luteal and random-start double ovarian stimulation protocols, may also use progestins to inhibit the endogenous LH surge. Women with PCOS or high responders also require the 'freeze for all' strategy and frozen embryo transfer in subsequent cycle and therefore may benefit from this approach. The cost of therapy and patient satisfaction score were significantly better in the PPOS group. However, long term financial superiority, pregnancy and neonatal outcomes remains to be seen.

Limitations reasons for caution

Neither the enrolled participants nor the OPD physicians were blinded to the study. As many patients were still pregnant and undelivered by the end of the study, the live birth rates were not studied in the follow-up. The dose effectiveness of Dydrogesterone was not assessed in this study but was used as per recommendations from previous studies.

Discussion and Conclusion

In this study, Dydrogesterone was the progestin used during COH as PPOS and its efficacy compared with GnRh antagonist Ganirelix acetate. The results of this study showed that DYG can be used as an adjuvant to hMG during ovarian stimulation to achieve comparable oocytes and embryos. It was effectively able to withhold premature LH surge and did not interfere with the measurement of endogenous progesterone levels. The pregnancy rates following FET in subsequent cycles in both the groups proved that DYG did not adversely affect the embryo quality or implantation rates.

Literature review shows that MPA, when used for PPOS causes stronger pituitary suppression and therefore these women required a much higher dosage of gonadotrophin and probably a longer duration of stimulation too [20]. Kuang., *et al*. compared hMG + MPA, 10 mg/d, with hMG + GnRH-agonist in the form of short protocol. They found longer duration of the stimulation and higher hMG dose in the MPA group compared to the short protocol (2014.0 ± 451.7IU vs. 1636.7 ± 659.6IU, $p < .005$) [20]. Zhu., *et al*. retrospectively compared IVF patients treated with hMG-short protocol with IVF patients treated with hMG and Utrogestan, 100mg twice a day [22]. They also found that higher dose of hMG was used in the Utrogestan group (1884.22 ± 439.47 IU vs. 1446.26 ± 550.48 IU, $p < .05$). In our study, patients were treated with the hMG + DYG there was no increase in the requirement of hMG dose or duration as compared to GnRh antagonist group. However, due to the differences in the progestin preparation, direct comparisons with the studies by Kuang., *et al*. and Zhu., *et al*. are not possible [20,22]. Dydrogesterone exerts a weaker effect on inhibition of GnRH than MPA. This could be due to the different GnRH secretion patterns regulated by different progestins. Intracellular progesterone receptors (PR) modulate the biological effects of progesterons at cellular level. The different progestins have different ability in binding to the progesterone receptors and hence their biological

results differ [23]. It is seen that the relative binding affinity of DYG towards progesterone receptors in circulation, is lower than that of MPA and probably similar pattern is exhibited at the hypothalamic level. However, further studies are needed to corroborate this hypothesis [23]. Progestin priming effectively downregulated folliculogenesis and increased ovarian sensitivity to exogenous gonadotropins [24-27]. These data would explain the no increased gonadotropin consumption in our study group.

In our current study, there was no case of premature LH surge and post trigger the LH levels rise (surge) was well documented and hence none of the cases was cancelled. Both the groups demonstrated a similar oocyte retrieval rates and metaphase II oocytes with a sufficient response. None of the study participants suffered moderate or severe OHSS.

Recently, Yu., *et al*. (2018) published the results of a prospective randomized clinical trial comparing MPA with DYG in a population of IVF patients under 36 years of age stimulated with HMG [28]. The number of oocytes retrieved (10.8 ± 6.3 for the hMG + DYG group vs. 11.1 ± 5.8 in the hMG + MPA group) were similar in both groups. There was no significant differences in the clinical PRs after the first frozen embryo transfer cycle (OR: 0.82, 95% CI: 0.56-1.21, p¼.33), 57.6% for the hMG + DYG group vs. 62.3% for the hMG + MPS group [28]. These figures are similar to the clinical pregnancy rate per embryo transfer found among frozen embryo transfer participants in our study (45.45% vs 37.27%, p = 0.07). Due to the limitation of small sample size, the results of this study in terms of clinical pregnancy rates, should be interpreted with caution. The total number of embryos obtained was also similar.

Unfortunately, due to the short follow up of the patients at the time of submitting this article, no definitive data about cumulative PRs and live birth rates of the entire cohort of study population could be assessed. Medroxyprogesterone acetate (MPA) was used by Kuang., *et al.* for its unique advantage it being a strong progesterone and has fewer androgenic properties (20). The synthetic progesterones did not interfere with the autogenous progesterone measurements. However, MPA was contraindicated in human pregnancy because MPA has dose-related teratogenicity and toxicity in animals, although inadvertent exposure to therapeutic doses does not appear to present a significant risk of structural defects [20,29]. Dydrogesterone is known to be safe in pregnancy and hence was used in this study.

We could not study and titrate the dose of DYG and calibrate the extent of suppression of the HPO axis as it was beyond the scope of this study. We used 30mg of Dydrogesterone per day in group 1, starting from day 2 of stimulation, as previous studies have shown that 30mg dose maybe the effective dose, although further evidence is needed to verify this.

The use of progestins to prevent the LH surge in ovarian stimulation cycles

It has been presumed that those pituitary glands secretions could have been transiently suppressed by high doses of progesterone during luteal phase ovarian stimulation. This supposition is in agreement with Letterie's study showing that a combination of ethinyl oestradiol and norethindrone administered for 5 days beginning on day 6 or 8 of the menstrual cycle permitted folliculogenesis, but inhibited the mid-cycle LH surge and consequently ovulation during ovarian stimulation [30]. The modern technology of vitrification allows safe cryopreservation of oocytes and embryos with a post-warming survival rate very close to 99%. Hence the transfer of fresh embryos to a uterus that has been newly subjected to hormonal stimulation is no longer required. The appropriate inhibition of the LH surge with exogenous progesterone shows that progestins could be an possible substitute to GnRH agonists and antagonists for preventing LH surge during COH in IVF/ ICSI cycles.

The first study on the use of a progestin during ovarian stimulation was published by Kuang and colleagues in 2015 [20]. The authors tried to use MPA in preventing LH surge, and further compared cycle characteristics along with pregnancy outcomes in subsequently frozen thawed embryo-transfer cycles, against short protocol as control group. MPA was used as an alternative to progesterone for its advantages: it is progestative and slightly androgenic, and does not interfere with the measurement of endogenous progesterone production. The number of oocytes retrieved in the study group was slightly higher than in the short protocol, although the difference did not reach significance, which was similar to our study. The mean duration of stimulation and HMG dose were significantly higher than in the control group, unlike in our study where both groups were similar. The oocyte maturation rate, fertilization rate or cleavage rate was similar between the two groups as in our study. Further, the number of good-quality embryos and cryopreserved embryos were also

similar between the two groups. Also as with previous studies none of the study participants suffered moderate or severe OHSS. The incidence of premature LH surge was similar in both the study and control group (0.7% versus 0%). The clinical pregnancy rates, implantation rates and live birth rates were also similar in both the study groups. The results of the study provided first-time evidence that MPA is an effective oral alternative for the prevention of premature LH surges in women undergoing ovarian stimulation, and the pregnancy outcomes from frozen-thawed embryo transfer cycles indicated that the embryos originating from this regimen had a similar development potential to those from the control group.

Similar study is seen in women with polycystic ovary syndrome (PCOS), who underwent COH for IVF. In this prospective controlled study MPA was compared with a short protocol [21]. Women with PCOS planning to have an IVF represent a therapeutic challenge: they are predisposed to poor oocyte quality, low fertilization rates, high miscarriage rates and risk of OHSS. The fertilization rate and ongoing pregnancy rate per transfer in the study group were higher than those in the control group (77.69 ± 16.59% versus 70.54 ± 19.23%, $P < 0.05$; 58.67% versus 42.86%, $P < 0.05$), unlike our study wherein both groups were comparable. Two cases of OHSS were reported in the short protocol group, while we did not have any case of OHSS in our study. However, this needs to be viewed with caution due to the small number of study participants.

Some studies have reported concerns about prolonged exposure of the developing follicles to progesterone. Although previous studies and a metaanalysis [31,32] have shown that progesterone elevation in the late follicular phase has no adverse effect on oocyte and embryo quality, suggesting that elevated exposure of the developing follicles to progestins is safe, several recent publications have challenged this concept. Elevated progesterone concentrations on the day of oocyte maturation induction have in fact been said to significantly reduce the formation rate of top-quality blastocysts. Progesterone elevation on the day of HCG administration has been said also to adversely affect the cumulative live birth rate per oocyte retrieval cycle, even if this result seems more dependent on the detrimental effect of progestin on the endometrium.

Progestins other than MPA have also been explored in PPOS protocols by Zhu., *et al.* [22]. Conducted under the same conditions as for MPA, a retrospective study compared Utrogestan taken orally in the form of soft capsules (200 mg/day) with a short protocol. Despite the higher amount of HMG (1884.22 ± 439.47 IU versus 1446.26 ± 550.48 IU, $P < 0.05$), the number of mature oocytes was not significantly different in these groups of normal responders. Women in the Utrogestan group had significantly higher number of viable embryos as compared to the short protocol ($P < 0.05$), although the ongoing pregnancy rate was not significantly different. In an attempt to test new synthetic progestins that represent the most suitable option for PPOS, Dydrogesterone (DYG) was used in our study. The results of our study showed comparable oocyte retrieval and viable embryo numbers in the two groups, with similar pregnancy outcomes. Dydrogesterone was able to effectively inhibit premature LH surge, without affecting measurement of endogenous progesterone.

Other than in women seeking fertility preservation, PPOS may be proposed as a first-choice protocol in all conditions where ovarian stimulation and oocyte retrieval are not followed by a fresh embryo transfer like donor stimulation, PGT-A (preimplantation genetic testing for aneuploidies) and PGT-M (preimplantation genetic testing for monogenic/single gene defects) cycles, dual ovarian stimulation (and non-conventional protocols) and in women at risk of OHSS.

One important advantage of the association between a progestin and FSH/HMG in high responders is that the triggering may be exerted by the GnRH agonist, which helps to avoid early-onset OHSS. In addition, cryopreservation of all embryos with delayed transfer can diminish the risk of late-onset OHSS. Other advantages over the use of a progestin in preventing the LH surge are oral administration, easier access and more control over LH concentrations [21]. This programme is also more patient-friendly as fewer injections are required and it is much cheaper.

In our study, DYG group cycles had a total cost of medication significantly lower than Ganirelix cycles. As per the financial analysis, COH with PPOS reduces the overall cost of the treatment along with the cost per retrieved and effective oocyte. We believe that cost savings are important, but so are patient comfort and compliance, and clinical results among recipients. The cost savings jointly with the higher degree of comfort and satisfaction of patients with this new protocol deserve further studies to confirm these promising results. Nevertheless, more research and studies

are needed with randomized control trials involving larger number of patients to confirm the suppression of LH surge and the other embryological and clinical outcomes.

Despite these advantages, progesterone-blocking strategies associated with delayed embryo transfer may have some weaknesses.The patients need to return and be rescheduled for cryopreserved embryos transfer. Data on subsequent cryopreserved embryo transfer are still limited. Those protocols furthermore require a change in the practice of current IVF programmes, a good cryopreservation programme, and further evaluation on medical and economical aspects, as many of the conclusions are based on retrospective studies with limited number of patients.

Conclusions

The application of progestins for inhibiting ovulation in ovarian stimulation cycles for IVF has been shown to be effective and safe, with good results reported in terms of the number and quality of the oocytes and embryos. The main clear advantage of this new form of treatment is its simplicity and hence it is patient friendly. This is not only due to a major reduction in the number of injections, but also in the need for monitoring controls. Because there is no fear as to when to introduce the antagonist according to the leading follicle size, and there is no need for gonadotropins dose modification, the first monitoring visit can be performed on the eighth stimulation day, and likely, the trigger day can already be foreseen. The large scale application of PPOS could be revolutionary for several reasons. With the growing use of IVF, it is preferable to make the treatment as convenient as possible for the patients, possibly converting the route of administration from subcutaneous injections to oral intake. The cost of progestin compared with GnRH analogues also seems extremely beneficial. The main limitations of this study are being a small study from a single centre, the sample size is not very large and the short follow-up. Therefore, we recommend future studies on endocrinology, reproductive, obstetric and neonatal outcomes, before this protocol can be recommended widely.

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