Soft versus Conventional Protocol in Ovarian Stimulation in Intracytoplasmic Sperm Injection Cycles for Poor Responders: A randomized clinical trial

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Abstract

Background: Mild(soft, minimal) protocols have been proposed as an alternative to the classical protocols , a controlled ovarian stimulation is defined as "soft" either when (a) Gonadotrophins (Gn) are given at a lower dose and/or for a short period together with a GnRH-antagonist, or when (b) oral compounds (e.g. anti-estrogens) are used alone or in combination with Gn and GnRH-antagonists.

Objectives: The aim of this study was to compare between soft and conventional protocol in ovarian stimulation for poor responders women undergoing Intracytoplasmic sperm injection procedure(ICSI),

Patients and methods: This study is A randomized clinical trial, carried out at Assisted Reproduction Unit, Qena University hospital, South Valley University, Egypt, on 120poor responder women undergoing ICSI divided randomly into two groups:(Group 1): included 60 patients received soft ovarian stimulation protocol, (Group 2): included 60 patients received conventional ovarian stimulation protocol,

Results: In comparison between the two groups regarding cancellation rate ,chemical and clinical pregnancy were comparable with no statistically significance between soft group and conventional group.

Conclusion: This analysis presents strong evidence in favor of soft stimulation in intracytoplasmic sperm injection cycles for poor responders, which therefore should currently be considered a treatment of choice for patients requiring intracytoplasmic sperm injection cycles for poor responder's treatment.

Key words: soft , conventional , poor responder, ICSI.

Introduction

Age and low number of antral follicle count are the biggest difficulties that face intracytoplasmic sperm injection procedure (ICSI) nowadays. **(Bastu et al., 2016)**. ESHRE Conesus chose multiple characteristics (Bologna) to determine low responder. Bologna criteria: At least 2 of 3 criteria to define poor responder:

i. Age is Forty years or more.
ii. A previous POR (≤ 3 oocytes with a conventional stimulation protocol).
iii. An abnormal ovarian reserve test at least one of these tests (AMH less than 1.1 ng/ml , low AFC <7) **(Ferraretti et al., 2011)**

The cornerstone for induction for low ovarian reserve patients is larger dose of gonadotropins. The higher Gn dose the more cost of IVF that would be acceptable if there was increase in IVF success. But, many studies suggest that number of oocytes may increase with higher doses of gonadotropins, but not the clinical pregnancy rate **(Revelli et al., 2014)**.

The era of use of GnRh antagonist in assisted reproduction technology has allowed to use low doses of stimulation protocols for IVF treatment. During mid cycle GnRh antagonist was injected before LH rise so this allows for starting ovarian induction for the IVF cycle without affection number of follicles recruited in follicular phase. This allows FSH secreted by pituitary to rise to help in follicles recruitment, this lead toa reduction of exogenous hormones used**(Verberg et al., 2009)**.

Soft (mild, minimal) ovarian induction protocols include natural cycle, modified natural cycle or mild ovarian induction. Soft induction had been used for women with Bologna criteria. .**(Labarta et al., 2018)** .Mild protocols is a new protocol used for poor responder, according to ISMAAR association, a controlled ovarian induction is known as "soft" either (a) exogenous gonadotropins are given at a small dose or when given a short period together with gonadotropin releasing hormone-antagonist,

or when (b) oral compounds are given with injectable doses of gonadotropins and gonadotropin releasing hormone-antagonist **(Revelli et al., 2014)**.Soft protocols have a less impact on the ovary, are easy to use, have less side effects, are quicker and cheaper as low amount of drugs used and for short time**(Revelli et al., 2014)**.

Aromatase inhibitor has a golden role in stimulation protocols with fewer drawbacks than estrogen receptor blocker. It does not cause endometrium atrophy so it is suitable for embryo implantation, increase response follicles to grow to gonadotropins and less incidence of ovarian hyper stimulation and multiple pregnancies**(Verpoest et al., 2006)**.

The purpose of this trial was to discriminate efficacy of soft and conventional protocol in ovarian induction for low ovarian response women undergoing ICSI procedure.

Patients and methods

This was a randomized clinical study, where patients had been attending Assisted Reproduction technology Unit, Qena University hospital, South Valley University, Egypt.

Inclusion criteria

Women hadlow ovarian reserve undergoing ICSI procedure (Bologna Conesus) **(Ferraretti et al., 2011)**.

Exclusion criteria

Hyper or Normal responder's patients. Hyperprolactinemia , thyroid dysfunction, DM and adrenal disorder**.** Patients with severe male factor .women with renal dysfunction, liver dysfunction and systemic lupus disease.

Methods: All Patients were subjected to:

History taking

Included the duration of infertility, previous ovarian surgery as cystectomy and the male factor, history of previous ICSI or IVF trials.

Examination & Laboratory investigations

General, abdominal and vaginal examination.: FSH, LH (basal), Prolactin, AMH, basal Estradiol (E2), Thyroid Stimulating Hormone (TSH).Hormones level FSH, LH, oestradiol, and progesterone level were measured by ELISA kits by Mini-Vidas technique with a sensitivity of 0.2 ng/ml (measurement range was 0.2-40 ng/ml).

Evaluation of male factor: Husband semen analysis to exclude azoospermia**,** severe male factor.

Uterine cavity assessment: Done by transvaginal 3-dimentional ultrasound [Medison sonoacex8-3d Transvaginal probe 6.5MHZ] or office hysteroscopy in the cycle prior to ICSI cycle.

Samples: poor responders women attended in ART unit in Obstetrics and Gynecology department, South Valley University hospital from May 2019 to August 2020.

Patients participated, and fulfilled the inclusion, after signed the informed consent form, were classified randomly in two groups by using closed envelope (serial number): Group **|**:who received soft ovarian stimulation protocol. Group **‖**: who received conventional ovarian stimulation protocol.

At cycle day 2 estradiol concentration≤ 50pg/ml and vaginal ultrasound scan[GE logiq p5 transvaginal probe 4 to11 MHZ] were done to detect any residual ovarian cyst.In **group |** : At cycle day 2 Ovarian stimulation began with Oral tablet letrozole 2,5 mg/two times per day [Letrozole , Acdima]for five days and 150 IU of menotrophin intramuscular (Menopur , Ferring) daily till time of trigger. In **group II**: Ovarian stimulation with [300-450IU] gonadotrophins divided into150iu recombinant FSH intramuscular [Gonal F, Merck] and (150-300) highly purified menotrophin intramuscular (Menopur, Ferring)was started on Cycle Day2 of menstrual cycle. The Dose of Gonadotropins was adjusted according to response.

For both groups

Vaginal ultrasound [GE logiq p5 transvaginal probe 4 to11 MHZ]was used to assess follicular maturation. For both groups antagonist suppression was used. Cetrorelix acetate 0.25mg/day subcutaneously [Cetrotide, Merck] was given and continued until time of triggering when follicles reached 14 mm. When one or two follicles achieved 18 mm or more in mean diameter in one or both ovaries , final oocytes maturation was performed with injecting 500 microgram of recombinant HCG subcutaneously [Ovitrelle 250µg/.5ml, Merck, Serono, Inc].The cycle was cancelled for inadequate response. If there was no growing follicles after 7 days of stimulation.

Oocytes retrieval was done 36 hours later on post triggering under general anaesthia using suction apparatus with pressure 100 mmhg. Intracytoplasmic sperm injection (ICSI) procedure was done for all cases in both groups. Fresh embryo transfer was done if endometrium was appropriate (8-12mm triple). we recorded in each patient oocytes retrieved, their quality, fertilized oocytes,number of embryos and their degree.

Embryo transfer

Only 1-2 of [day3 to day5] embryos were transferred after progesterone supplementation100 mg daily intramuscular (Prontogest ampoules, IBSA) using ultrasoft embryo transfer catheter (labotect or walace) under abdominal ultrasound guidance [GE logiq p5 –trans abdominal probe1.6-4.6MHZ].

Luteal phase support: All patients were given daily intramuscular progesterone 100 mg (Prontogest ampoules, IBSA) from the day after ovum retrieval till time of HCG testing. Serum β HCG level was assessed on day 14 after ET and considered positive if >5 MIU/ML, progesterone support continued in case of positive HCGtest till 12 weeks gestation.

Follow up: Quantative β -HCG after two weeks from day of embryo transfer :Fetal heart pulsation.

Ethical Considerations

Dealing with data and data dissemination was confidential. Women were informed by Statement describing the study protocol .All women signed a written Informed consent before starting the study with counseling about risk and benefit of study. Well qualified and trained personnel conducted the research. The consent form was provided with the proposal. Ethical committee of the Faculty of Medicine –South Valley University reviewed and approved proposal.

Statistical Analysis

SPSS program software version 26.0, Microsoft Excel 2016 and MedCalC program software version 19.1 are used for analyzing collected data. Numerical parametric data were analyzed by descriptive statistics as mean±SD (standard deviation) and minimum & maximum of the range and for numerical non parametric data as median and 1st& 3rd inter-quartile range, while they were done for categorical data as number and percentage. Quantitative variables were analyzed by inferential analyses using independent t-test in cases of two independent groups with parametric data and Mann Whitney U in cases of two independent groups with non-parametric data. Qualitative data were analyzed by inferential analyses using Chi square test for independent groups. The level of significance was taken at P value <0.05 is significant, otherwise is non-significant. The p-value is a statistical measure for the probability that the results observed in a study could have occurred by chance.

Results

There were no significant differences between groups in consideration to Age, BMI, duration, type and cause of infertility, (**Table .1).**

There were a statistically significant differences between groups regarding Total dosage of Gonadotropin, Number of mature follicles, Endometrial thickness, number of oocyte and m2 oocyte, but there is no statistically difference between groups in consideration to number of days of stimulation, **(Table 2,3)**.

There were no statistically differences between groups regarding oocyte fertilized, good quality

embryos, transferred embryo, cancellation rate (due to no growing follicles, empty follicles, failed fertilization) ,chemical pregnancy , and clinical pregnancy, **(Table 4)**.

Table 1.Comparison between group| and group II as regard to age, BMI, duration, type and cause of infertility

Variables		**Group |** (n=60)		**Group II** (n=60)		**T**	**P**
Age (years) Mean ± SD		38.23± 2.47		38.47± 2.30		0.097	0.923
BMI (kg/m^2) Mean ± SD		27.07± 3.07		26.90± 3.54		0.897	0.369
Type of infertility No%	**Primary**	48	80.0%	50	83.3%	0.223	0.637
	Secondary	12	20.0%	10	16.7%		
Causes of infertility No%	**Male causes**	18	30.0%	26	43.3%	10.79	0.056
	Ovarian causes	10	16.7%	4	6.7%		
	Tubal causes	8	13.3%	10	16.7%		
	Peritoneal causes	2	3.3%	2	3.3%		
	Combined causes	16	26.7%	6	10.0%		
	Unexplained causes	6	10.0%	12	20.0%		
Duration of infertility	**Mean± SD**	6.23± 1.87		6.37± 1.62		0.447	0.655

Table 2. Comparison between group| and group‖ as per AFC, AMH and E2 basal level

Variables		**Group |** (n=60)	**Group II** (n=60)	**T**	**P**
Antral follicle Count	**Mean± SD**	4.77± 1.06	5.13± 1.07	1.65	0.100
AMH ng/dl	**Mean± SD**	0.79± 0.30	0.75± 0.30	0.817	0.414
E2 basal pg/ml	**Mean± SD**	41.12± 6.65	40.53± 6.31	0.510	0.610

Table 3. Comparison between group| and group II as per ICSI characteristics

Variables		Group I (n=60)	Group II (n=60)	T	P-value
Total dose of gonadotrophin (IU)	**Mean± SD**	1795.0± 341.57	4632.5± 1289.51	9.47	**<0.001**
Duration of stimulation in days	**Mean± SD**	11.97± 2.28	11.47± 1.68	1.49	0.137
Number of mature follicles	**Mean± SD**	5.97± 2.22	7.17± 2.53	2.39	**0.017**
Endometrial thickness mm	**Mean± SD**	8.17± 1.38	9.37± 1.55	4.28	**0.05**
number of oocyte	**Mean± SD**	3.20± 2.15	4.40± 2.82	2.19	**0.028**
m2 oocyte	**Mean± SD**	2.30± 1.75	3.47± 2.83	2.27	**0.023**

Table 4. Comparison between group| and group‖ as per ICSI process

Variables		Group 1 (n=60)		Group II (n=60)		T	P
Fertilization rate	**Mean± SD**	2.07± 1.22		2.73± 2.02		1.61	0.108
Number of embryos and grading(modified gardner system) 1-A or B 2-C and D No%		25(41%) 35(59%)		27(45%) 33(55%)		10.71 9.76	0.152 .23
Transferred embryo	**Mean± SD**	1.47± 0.77		1.50± 0.89		0.239	0.811
Cancellation rate No%	**Cancelled**	8	13.3 %	10	16.7%	0.261	0.609
Chemical pregnancy No%	**Positive**	10	16.7 %	14	23.3%	0.833	0.361
Clinical pregnancy No%	**Positive**	8	13.3 %	12	20.0%	0.960	0.327

Discussion

Ovarian hyper stimulation is cornerstone in ICSI procedure, as it induces multiple follicles growth,

leading to a higher number of oocytes retrieved and higher number of embryos, so lead to more success to get pregnant (**McCulloh et al., 2019**). Women with low ovarian reserve represent large section of women seeking for ICSI, they had a big problem due to reduced number of oocyte retrieved, increased cancellation rates and decreased pregnancy rates. The most suitable protocol used in ovarian stimulation for poor responders is to tailoring dose to each patient, based on AFC and AMH. Ferraretti et al. presented the Bologna criteria in order to determine a definition for poor responder. Among the various protocols, there was no evidence on the effectiveness of any one stimulation protocol over another (**Conforti et al., 2017**). GnRh agonist and antagonist have pregnancy and cancellation rate but some studies clarified advantage of the flare-up over the letrozole/antagonist protocols. A recent comparison among GnRH-agonist protocols, clarified a advantage of long GnRH-agonist protocol over the short GnRH-agonist protocol in consideration to number of clinical pregnancy, number of oocytes retrieved, and cancellation rates (**Siristatidis et al., 2015**). Mild ovarian stimulation protocols using low doses of gonadotropins have significant advantages, including cost effectiveness, although low number of expected oocytes retrieved. This has low success for poor responder, where the number and quality of the embryos is decreased. Clomiphene citrate together with letrozole is one of the main drugs used in soft protocols for ovarian stimulation of poor responders.(**Kolibianakis et al., 2015**).

In our study, comparison between the two groups regarding age, BMI were comparable with no statistically significance difference between soft group and conventional group .Our results were in line with trial of **Siristatidis et al., 2017** as they clarified that there was no statistically significance between their groups regarding age and body mass index. Fifty-eight patients participated in this study. 33 were received a mild protocol .the other twenty five received long GnRH-agonist protocol or GnRH-antagonist protocol.

In our research, comparison between the two groups regarding duration, type, cause and number of previous trials were comparable with no statistically significance difference between soft group and conventional group. Our results were in line with study **Yucel et al., 2014** as they published that there were no significant difference between two groups in consideration to age of patients and period of infertility. Also, **Xi et al., 2020** revealed that age, body mass index and period of infertility were the same between groups (P>0.05).

In our trial, comparison between two groups regarding Antral follicle count, AMH and E2 basal it showed that there was no statistically significance difference between the two groups .Our results were supported by study of **Siristatidis et al., 2017** as they reported that there was no statistically significance between their groups regarding Antral follicle count , AMH.

In our study, comparison between two groups regarding all dosage of gonadotropin , mature follicles number, endometrial thickness and number of M2oocytes were comparable with statistically significance difference between soft group and conventional group .

In our research, comparison between the two groups regarding duration of stimulation and maturation index was comparable with no statistically significance difference between soft group and conventional group.

In our trial, comparison between the two groups regarding fertilization rate, number of good quality embryos and transferred embryos were comparable with no statistically significance between soft group and conventional group. Our results were supported by study of **Siristatidis et al., 2017** as there was statistically significance between two groups in number of follicles in diameter 14-15mm and 18mm but 16-17mm there was no significance. Similarly, they found significant difference between two groups in number of COC and M2 oocyte [1 (95% CI=0-4) vs. 3 (95% CI=0-8.4), p<0.001 and 1 (95% CI=0-4) vs. 2 (95% CI=0-7.4), p=0.001, respectively], and, they found statistically significance regarding to fertilization rate, fresh and frozen embryos (all p-values <0.05) in control group. There was statistically significance regarding to endometrial thickness at day of trigger as it was lower in the study group compared to the control group [7.8 (95% CI=4.1-12) vs. 10 (95% CI=5.1-13.6) mm, p=0.015] and there was statistically significance regarding to cancelled

cycles rates as they were more in study group [36.4% (95% CI=19-53.7) vs. 12% (95% CI=1.7-25.7), p=0.036]. There were no failed fertilization. Furthermore, **Yucel et al., 2014** revealed that duration of stimulation was not statistically different. oocytes retrieved number were significantly higher in (estradiol + progesterone/letrozole + gonadotropin and GnRH antagonist) group (1.7 ± 0.7 versus 2.6 ± 0.6).Also, **Labarta et al., 2018** demonstrated that a significantly larger doses of gonadotrophins were used in the conventional ovarian stimulation protocol with a 3.6-fold increase (P < 0.05). There was significant higher oocytes retrieved number, (MII), number of fertilized oocyte and number of good-quality embryos in the minimal ovarian stimulation (MOS) protocol, and each parameter increased by 55.4% (P = 0.002), 63.7% (P = 0.001), 94.9% (P = 0.0005) and 326.7% (P = 0.002), respectively. Among the 30 patients who underwent a COS cycle first, the mean of good-quality embryos per cycle was 0.30 (9/30) in the COS cycle compared with 1.13 (34/30) in the MOS cycle (P = 0.001). On the other hand, in those 16 patients who underwent MOS first, the mean of good quality embryos per cycle was 0.31 (5/16) in the COS compared with 0.69 (11/16) in the MOS cycle.

In study conducted by **Matsaseng et al., 2013**, they show that conventional protocols has statistically significance in regard to oocyte retrieved (p = 0.000) and mild protocol had significant difference in regard to low doses of gonadotropin (p = 0.000). According to **Ashrafi et al., 2018**, there was significant difference in regard to total dosage of gonadotropins (P < 0.001), number of days of stimulation (P < 0.001), number of ovum picked up (P = 0.01) and embryo with high quality (P < 0.001) , cancellation (P = 0.002) and fertilization rates (P = 0.002)between groups. While in the study of**Yarali et al., 2009**, there was significant decrease in the total gonadotropin consumption, number of days of stimulation, and oocytes retrieved with soft stimulation in comparison with flare up agonist stimulation. The number of doses of gonadotropins were higher in control group than study group and this finding is in line with previous published trials, that utilized clomiphene citrate for their mild regime. The more dose of gonadotropins the more mature follicles and mature M2, this matched with clinical trials that published, while others report similar and one lower rates (**Mashayekhi, &Karimzadeh, 2013**).cancellation rate increased with mild protocol with clomiphene citrate In addition, mild stimulation with clomiphene citrate due to failure of ovum pick up, as also published in two clinical trials (**Fujimoto et al., 2014**&**Revelli et al., 2014**), whereas in the remaining two, there was no significant difference in cancellation rates between groups (**Mashayekhi, &Karimzadeh, 2013**, **Youssef et al., 2011**).

Fertilized oocyte rates were good and nearly equal with two protocols that were given, good fertilization rate in all cases of ICSI reflect good quality of the Embryology Laboratory (**Siristatidis et al., 2017**). This result is founded in previous study (**Revelli et al., 2014**). Endometrium in patients who received conventional protocol was more convenient than other group as clomiphene citrate could cause endometrium atrophy, a result that was matched to those of the largest RCTs (**Mashayekhi and Karimzadeh, 2013**). High total dosage of gonadotropins in the control group led to more number of embryos and transferred embryos. These results were matched with those of the largest RCT conducted (**Revelli et al., 2014**), while the rest report similar (**Mashayekhi and Karimzadeh, 2013**) or even lower (**Youssef et al., 2011**) numbers of embryos.

In our trial , comparison between the two groups regarding cancellation rate ,chemical and clinical pregnancy were comparable with no statistically significance between soft group and conventional group. Our results were supported by study of **Siristatidis et al., 2017** as they reported that there was no difference between groups with regards to positive pregnancy test [15.2% (95% CI=2.2-28.1) vs. 20% (95% CI=3.1-36.9), p=0.628], clinical pregnancy [12.1% (95% CI=4-23.9) vs. 20% (95% CI=3.1-36.9), p=0.412], and live birth [9.1% (95% CI=1.3-19.4) vs. 12% (95% CI=1.7-25.7), p=0.719] rates, and abortion [40% (95% CI=28-100) vs. 40% (95% CI=28-100), p=1.000], trial could not detect differences as it was low powered.

In the study of **Labarta et al., 2018**, of the 46 patients, 26 did not have embryo transfer because no oocytes or embryos were available (n =13) or all embryos had genetic disorder (n = 13). Finally, 20 patients underwent embryo transfer. Thirteen of them were carried out with embryos from soft protocol cycles. Three of the women experienced pregnancy losses (two only positive pregnancy tests and one clinical abortion) and one had a live birth. Four patients received only embryos obtained after conventional protocol cycles; two of them had a live birth. Finally, three cases were mixed transfers (1 embryo derived from MOS and 1 embryo from COS), resulting in two live births (1 single and 1 twin pregnancy). Result in four single and one multiple pregnancy with totally six live babies. Only 53.8% of the MOS transfers were carried out with good-quality embryo grade A or B (seven out of 13), compared with 100% in COS transfers (four out of four) and 66.6% in mixed transfers (two out of three).

Also there was agreement with trial of **Yucel et al., 2014**,there was no significant difference according to total dosage of gonadotropin , E2 level at time of trigger, embryos transferred, fertilized oocytes rate, implanted embryos, clinical pregnancy rate and live birth rate between groups (p> 0.05).While in the study of **Matsaseng et al., 2013**, standard protocol had higher significant difference on live birth rate [70/444 (15.7%) soft protocol vs. 78/325 (24%) standard protocol] (OR 0.59, CI 0.41-0.85, p = 0.004). Higher continuing pregnancy were observed [140/696 (20%) soft protocol vs. 144/547 (26%) in standard protocol] (OR 0.72, CI 0.55-0.93, p = 0.01).

Conclusion and recommendations

This study gives evidence in direction of Soft protocol in ICSI cycles for poor ovarian reserve, which therefore should be a first line for women with low ovarian reserve requiring ICSI cycles .In the future, more data on LBRs in both mild and conventional stimulation IVF is still required for proper and accurate comparison. Large randomized controlled trial is still required for a further substantial analysis of the cost-efficiency of co-treatment of letrozole in ovarian stimulation cycles. More patients, longer follow-up, and multicenter experience are all necessary to accurately figure out the role of conventional protocol in ovarian stimulation for poor responders' women. Further studies on large geographical scale and on larger sample size to emphasize our conclusion.

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