The Clinical Significance of a Low Percentage of Mature Oocytes Retrieved using Common Ovarian Stimulation Protocols.

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Abstract

The objective of this study was to investigate whether percentage of mature oocytes retrieved from ovaries stimulated with long agonist or multi-dose antagonist protocols affect the implantation, clinical pregnancy and live birth of ICSI (Intracytoplasmic sperm injection) cycles. The 654 cycles of agonist (long lupron) and 610 cycles of multi-dose flexible antagonist (antagon) were analyzed after stratification according to the percentage of the mature oocytes retrieved. The clinical pregnancy of the groups with less than 30 % mature oocytes retrieved, both antagonist and agonist protocol was statistically lower (at least p< 0.05) compared to the groups with more than 30% mature oocytes retrieved. In the agonist protocol, the implantation and live births for this group were significantly (p<0.009) lower than in the group with ≥70% mature oocytes retrieved. The live births in groups with more mature oocytes retrieved (30-69% and ≥70 %) of the antagonist protocol were lower (22.2% vs. 35.9% and 23.9% vs. 41.5%, p<0.0001, respectively) compare to the agonist protocol.

The results of our study showed that a very low percentage of mature oocytes retrieved impacts the clinical outcome of antagonist and long agonist protocols.

Introduction

Since the early 1980's a variety of controlled ovarian hyperstimulation (COH) protocols with the use of gonadotrophin-releasing hormone (GnRH) analogues (agonists or antagonists) in conjunction with gonadotropins/menotropins have been developed [1-4]. The addition of GnRH analogues significantly reduced the incidence of premature LH surges and cycle cancellations, leading to a higher number of oocytes retrieved, with an improved outcome of infertility treatments [5-8]. Despite a significant number of studies [3, 12, 13, 31, 34, 38, 40, 42, 48, 52] comparing both long GnRH agonist and GnRH antagonist protocols, the superiority of one over another is still widely debated in the literature.

The mechanism of action of these two analogues (agonist and antagonist) is different. In the long agonist protocol, antral follicles are recruited by exogenous gonadotropin after early depletion of endogenous hormones. In the antagonist protocol the endogenous hormones recruit follicles. GnRH antagonist controls gonadotropin secretion by its immediate suppression in the pituitary. The use of the antagonist is associated with a shorter time of stimulation, reduced gonadotropin consumption and a reduced ovarian hyper-stimulation incidence (OHSS) as well as a different pattern of steroid hormones concentration in the blood and follicular fluid [9], when compared to the agonist protocol [10-14]. The differences in the endocrine environment for maturing oocytes may alter the ovarian folliculogenesis and influence the oocyte quality [15-17] and subsequently embryo development [18]. It has been demonstrated that embryos derived from the women stimulated with the flexible GnRH antagonist protocol underwent faster with the earliest cleavage than embryos derived from women stimulated with a long GnRH agonist protocol [19].

Previously, a positive correlation between the follicle size, oocyte maturity and pregnancy rate of women undergoing IVF, has been reported [20-25]. Others [26-28] demonstrated that larger leading follicles yield better pregnancy rates, but not necessarily a higher percentage of mature oocytes or availability of embryos for transfer [25]. Teissier et al. [17] showed a discrepancy between the size and maturity status of the oocytes collected from the patients stimulated with the agonist protocol. They found mature oocytes were present in smaller follicles, and immature in larger follicles. Nogueira et al showed a significantly higher percentage of immature oocytes were retrieved from large follicles in antagonist cycles when compared to the agonist protocol [27]. They found also a greater heterogeneity in maturity of oocytes retrieved from patients stimulated with antagonist. Therefore, taking into consideration the information available in the literature, we undertook the present study to determine whether a low, medium or high percentage of mature oocytes retrieved may influence the clinical outcome of the two commonly used ovarian stimulation protocols (flexible multi-dose antagonist and long agonist) in IVF patients.

Materials and Methods

The study was approved by the Institutional Review Board of the Abington Memorial Hospital, PA. The data of 1264 ICSI cycles (2000 – 2013 years) with information on the nuclear maturity of retrieved oocytes, were extracted from the electronic database. Any types of donors (oocytes, sperm) or gestational carrier cycles were excluded. The follicular growth was stimulated using common IVF protocols either agonist (long lupron, 654 cycles) or multidose flexible antagonist (antagon, 610 cycles) and pure gonadotropins and menotropins (Gonal-F, Serono Laboratiories INC. Norwell, MA, Follistim, Organon Inc, West Orange, NJ, Repronex and Brevale, Ferring Pharmaceuticals Inc., Tarrytown, NY). The ovarian stimulation started on day 3. The initial

dose of hormones was determined based on the follicles size, estradiol level in the blood, age of patient and type of diagnosis. Dosages were adjusted every 2-3 days in association with the follicles size and estradiol level in the blood. HCG 10,000 IU (Profasi, Serono, USA) was administered when at least one follicle was ≥18 mm in diameter, and endometrial thickness of >8 mm and appropriate estradiol levels achieved. The oocytes were retrieved 34-36 h later. To precisely assess the maturity of retrieved oocytes, denudation of oocytes using hyaluronidase (80 IU/ml; Conception Technology, San Diego, CA, USA) was performed 1-2 h after oocytes collection. Spermatozoa for the ICSI procedure was prepared using density gradient (90 & 45%; PureCeption, Conception Technologies, CA, USA) protocol. After injection, oocytes were cultured in a fertilization medium following cleavage media (both from Cooper Surgical Inc., Trumbull, CT, USA). Fertilization was assessed 17-20 h after injection. The embryo development (number and regularity of blastomeres, degree of embryo fragmentation and cytoplasm quality) was assessed daily. Ultrasound-guided embryo transfer (ET) was carried out on the day 3 after oocytes retrieval. The number of embryos transferred was in accordance to the guidelines of the Practice Committee of American Society for Reproductive Medicine (ASRM) and Practice Committee of the Society for Assisted Reproductive Technology.

Clinical Outcome.

Pregnancy was confirmed by determination of quantitative serum β hCG concentration on days 10-12 day and 12-14 after embryo transfer. The patients with a positive pregnancy test were evaluated 5-6 weeks later by ultrasound scanning of the uterus to determine the presence of gestational sac and/or fetal heart activity, or to diagnose ectopic implantation. The luteal phase was supported with a vaginal gel (Crinone 8%, Serono, USA) or micronized progesterone (100 mg/day)

until the 12th weeks of gestation. However, if the pregnancy test was negative, it was discontinued.

The patient's cycles both antagonist and agonist were stratified into three groups according to the percentage of the mature oocytes retrieved: group 1 ≤30% (n=19) for the antagonist and (n= 32) for the agonist; group 2 31 to 69 % (n=243) for the antagonist and (n= 251) for the agonist and group 3 ≥70% (n= 348) for the antagonist and (n=371) for the agonist. The percentage of mature oocytes was calculated from all oocytes retrieved. The aim of this classification was to develop groups with low, intermediate and high sensitivity of the ovaries to the stimulation protocols. Therefore, the cut-off points were developed based on our clinical experiences and visualizing the results. Patients with ≤30% mature oocytes retrieved were assigned to the group 1 (low ovarian sensitivity to the stimulation protocol). On average, 70-80% of retrieved oocytes are mature when the patients respond well to a stimulation protocol. This rationale led to creating group 3 (high ovarian sensitivity) and the intermediate group 2 (31-69% mature oocytes retrieved).

Statistical Analysis.

The results obtained were analyzed using Statistica 10.0 software (Statsoft, Tulsa, OK, USA and IBM SPSS Statistics 21.0, Predictive Solutions). Distribution of data was verified with the Shapiro-Wilk test and the Kolmogorov-Smirnov test with the Lilliefors correction. In view of non-Gaussian distribution of data, the non-parametric Mann-Whitney U test (a two group comparison) and the Kruskal-Wallis test (three groups comparison) were employed. The Pearson chi-square test was used to determine statistical differences in fertilization, clinical pregnancy, implantation and live birth rates among the studied groups and treatment protocols. The significance of correlations between the percentage of mature oocytes retrieved and cycle characteristics were examined by the Spearman (non-

parametric) test. Results of these analyzes were reported as median values with interquartile 25-75% in parentheses or percentage. P<0.05 was considered statistically significant.

Results

The distribution of diagnoses (endometriosis, idiopathic, male factor, ovulatory dysfunction, tubal, polycystic ovary syndrome, diminished ovarian reserve or advanced maternal age) in our antagonist and agonist protocol study groups is presented in Table I. The majority of patients in both treatment protocols were diagnosed as male factor (approx. 45%) and diminished ovarian reserve (approx. 25%).

The use of the antagonist or agonist in the ovarian stimulation protocols did not have any effect (p<0.35) on the overall percentage of mature oocytes retrieved. Percentage of mature oocytes retrieved correlated positively (p<0.003, data not shown) with the number of days of stimulation in both protocols. There were no differences between the three studied groups (≤30%, 31-69% and ≥70 %) of the antagonist and agonist protocols in relation to the age, day 3 FSH level, endometrium thickness, estradiol level on day of hCG, or estradiol concentration per retrieved oocyte (Table II). However, in the antagonist protocol treated group of ≥70 % matured oocytes retrieved, there was a tendency

(p<0.053) in enrolling more older patients. Consequently, the difference in the age (p<0.001) between the two treatment protocols of the groups with ≥70 % mature oocytes was established. The endometrium was significantly thinner in the antagonist protocol groups (p<0.004, p<0.001and p<0.002, respectively) compared to the agonist. The estradiol level at time of HCG injection was significantly (p<0.001) lower for the antagonist of second (31-69%) and third (≥70%) group compared to corresponding agonist groups (Table II).

There were no differences in the number of eggs retrieved between the antagonist groups. However, the first agonist protocol group (≤30% mature oocytes) had the lowest number of retrieved oocytes (p<0.04) when compared to other groups using this protocol (Table III). The number of mature and fertilized (2PNs) oocytes gradually increased (p<0.001) as more mature oocytes were retrieved in each of both protocol groups (Table III). There were no differences in the number of transferred embryos between each protocol group , as well as between the treatment protocols. There were no differences in the fertilization rate between the antagonist and agonist protocol groups (Table III). The differences between the treatment protocols (antagonist vs. agonist) were determined among the groups 31-69% and ≥70 % mature oocytes in the number of retrieved

Table I. Distribution of different diagnosis (endometriosis, idiopathic, male factor, ovulatory dysfunction [OD], tubal factor, ovarian failure, diminished ovarian reserve [DOR], polycystic ovary syndrome [PCO] or advanced maternal age [AMA]) in the studied groups of antagonist and agonist protocols. The values are presented as the percentage of patients with specified diagnosis within each group.

	% mature oocytes	Endom etriosis	Idio pathic	Male Factor	OD	Tubal Factor	Ovarian failure	DOR	PCO	AMA
Antagon	<30	0	0	0	25	0	0	0	0	0
	30-69	15	0	45	5	10	0	20	0	10
	≥70	7	0	40.5	7.1	14.3	0	35.7	2	11.9
Lupron	<30	14	0	42.8	8.6	14.3	0	31.5	0	8.6
	30-69	15.8	3.16	43.9	10.2	9.1	3.5	22.1	1	10.9
	≥70	18.6	2,2	48.9	9.5	10	7	17.1	1.9	11.7

Table 3. Antagonist and agonist cycle characteristics after stratification data according to the percentage of mature oocytes retrieved. Values are medians with interquartile 25-75% in parentheses.

Mature oocytes retrieved				
Antagonist - Antagon	**≤30 %** **n=19**	**31-69 %** **n=243**	**≥70 %** **n=348**	**P value**
Age (Years)	35.0 (32-36)	35.0 (32-38)	35.4 * (32-39)	NS
FSH (IU/ml)	10.0 (9-14)	9.8 (8.0-12)	9.9 (8-13)	NS
Endom. Thick. (mm)	9.5* (8-10)	10.0* (9-12)	10.0* (9-12)	NS
E₂ at hCG (pg/ml)	1604.5 (1299-1737)	1561.0* (1114-2028)	1517.0 * (1086-2012)	NS
E₂/oocyte (pg/ml)	156.7 (133-186)	161.7 (118.9-225)	165.9 (116-245)	NS
Agonist - Lupron	n=32	n=251	n=371	
Age (Years)	35.0 (32-37.5)	34.0 (32-38)	34.0 * (31-36)	NS
FSH (IU/ml)	10.3 (8-13)	9.2 (8-12)	9.5 (8-12)	NS
Endom. Thick. (mm)	11.0* (10-13)	11.0* (9-12)	11.0 * (9-12)	NS
E₂ at hCG (pg/ml)	1584.0 (1155-2234)	1915.0* (1354-2580)	1956.0* (1383-2572)	NS
E₂/oocyte (pg/ml)	157.4 (120-206)	152.3 (107-207)	161.8 (124-221)	NS

Note: NS = not statistically significant. The asterisks stand for the difference between the antagonist and agonist protocols. Specific p values are in the results section.

Abbreviations: FSH – follicle stimulating hormone, Endom. Thick. – endometrium thickness, E₂ – estradiol 17β, hCG – human chorion gonadotropin hormone

Table 3. Comparison of an average number of retrieved, matured, and fertilized oocytes and embryos transferred between the study groups and treatment protocols (antagonist and agonist). Reported values are medians with interquartile range (25-75%) in parentheses with an exception for the fertilization rate.

Mature oocytes retrieved				
Antagonist - Antagon	**≤30 %** n=19	**31-69 %** n=243	**≥70 %** n=348	**P value**
Number egg retrieved	9.0 (7-14)	10.0 * (7-14)	10.0 * (6-15)	NS
Number of M II	2.0 ᵃ (1-4)	5.0 ᵇ * (3-8)	8.0 ᶜ * (5-12)	0.001
Number of 2PN	1.0 ᵃ (1-2)	4.0 ᵇ * (2-5)	5.0 ᶜ * (3-8)	0.001
Fert. rate (%)	70.8 (50-100)	71.4 (50-86)	66.7 * (55-82)	NS
Number of embryo transf.	2.0 (1-3)	2.0 (2-3)	2.0 (2-3)	NS
Agonist - Lupron	n=32	n=251	n=371	
Number egg retrieved	8.5 ᵃ (7-13.5)	13.0 ᵇ * (8-19)	12.0 ᵇ * (8-18)	0.04
Number of M II	2.0 ᵃ (1-3)	8.0 ᵇ * (4-11)	11.0 ᶜ * (6-15)	0.001
Number of 2PN	1.5 ᵃ (1-2)	5.0 ᵇ * (3-8)	7.0 ᶜ * (4-10)	0.001
Fert. rate (%)	77.6 (66.7-99)	71.4 (55-87)	75.0 * (57-86)	NS
Number of embryo transf.	2.0 (1-3)	2.0 (2-3)	2.0 (2-3)	NS

Note: Different small letters stand for the differences between the groups within the antagonist and agonist treatment. The numbers in column P value show statistical significance level. NS = not statistically significant. The asterisks stand for the difference between the antagonist and agonist protocols (details are in the results section).

Abbreviations: M II – mature oocytes with the nuclear maturity of the metaphase II, 2PN – oocytes with two pronuclei, Fert. Rate – fertilization rate, Number of embryo transf. – number of embryo transferred.

(p< 0.0009 and p<0.0007, respectively), matured (p<0.0001, p<0.0004, respectively) and fertilized oocytes (p< 0.0004, p<0.0007, respectively). Additionally, the fertilization rate of the agonist group with ≥70% of mature oocytes was significantly higher (p<0.03) compared to the corresponding antagonist protocol group (Table III). The overall number of retrieved, mature and, fertilized oocytes was significantly higher (p<0.05) in the agonist protocol when compared to the antagonist protocol (data not shown).

There were no differences in implantation, and live births between the three studied antagonist protocol groups (Table IV). The clinical pregnancy rate (presence of sac and heart beat) in the first antagonist protocol group (≤30% mature oocytes) was lower (p<0.03 and p<0.05, respectively) when compared to the other two studied groups. In the agonist protocol, implantation and live births of the first group were significantly (p<0.009) lower than compared to third group but not the second group. The clinical pregnancy rate in first

agonist protocol group (≤30% mature oocytes) was lower (p<0.008 and p<0.023, respectively) compare to the other two groups. Live birth rates were significantly different between the antagonist and agonist protocols: 22.2 vs. 35.95% (p<0.0009) for groups 31-69% and 23.9 vs. 41.5% (p<0.0001) for groups with ≥70% mature oocytes.

Discussion

In our study, the groups with low ovarian response (less than 30 % of mature oocytes retrieved) of the antagonist (antagon) and the agonist (long lupron) were comparable in terms of characteristics assessed (endocrinological and embryological) and the clinical outcome (implantation, clinical pregnancy and live births). Similarly, Al-Inany et al. [29] showed no differences in clinical pregnancy rates between the GnRH antagonist and the GnRH agonist treatment in patients with low ovarian responses or PCO patients [30]. However, in our study, the groups with low

Table 4. Clinical outcome of the antagonist and agonist ICSI cycles in relation to the percentage of mature oocytes retrieved.

Mature oocytes retrieved				
Antagonist - Antagon	**≤30 %** n=19	**31-69 %** n=243	**≥70 %** n=348	**P value**
Implantation rate (%)	11.1	22.8	24.6	NS
Clinical preg. (sac) (%)	15.8 [a]	37.5 [b]	43.5 [b]	0.03
Clinical preg. (Htb) (%)	15.8 [a]	34.6 [b]	40.2 [b]	0.05
Live Birth (%)	10.5	22.2 *	23.9 *	NS
Agonist - Lupron	n=32	n=251	n=371	
Implantation rate (%)	13.5 [a]	22.6 [ab]	28.3 [b]	0.009
Clinical preg. (sac) (%)	18.8 [a]	42.6 [b]	46.9 [b]	0.008
Clinical preg. (Htb) (%)	18.8 [a]	41.0 [b]	43.7 [b]	0.023
Live Birth (%)	15.6 [a]	35.9 [ab]*	41.5 [b]*	0.009

Note: NS = not statistically significant. The column P value shows the level of statistical differences between the three studied groups. Values with different letters are different. The asterisks stand for the difference between the antagonist and agonist protocols (p<0.0009 for the groups 30-69% and p< 0.0001 for the groups with ≥70% matured oocytes).

Abbreviations: Clinical preg. (sac) – clinical pregnancy where the gestational sac was visible, Clinical preg. (Htb) – clinical pregnancy where the gestational sac and fetal heart beat were present.

percentages of mature oocytes retrieved from antagonist and agonist groups were significantly different in terms of the number of the matured (M II) and fertilized oocytes (2PNs) and clinical pregnancy from other studied groups. Additionally, in the agonist protocol, the implantation rate and live births of the <30% group were significantly lower from the group with the highest percentage of the mature oocytes retrieved. A low clinical outcome of the groups with lower number of mature oocytes retrieved might be a result of a lower sensitivity of follicles to ovulation induction protocols. It appears that this effect was more evident in the agonist than in the antagonist protocol, as the variables examined showed statistical differences. Thus, the same ovulation induction protocol might produce different treatment outcome in patients with different ovarian sensitivity to the stimulation protocol [29].

There were no statistical differences in clinical outcomes (implantation, clinical pregnancy and live births) between the second and third group (groups with more mature oocytes retrieved) of antagonist and agonist protocols. However, when the second and third group of antagonist was compared with corresponding group of agonist protocol, there were significantly lower live birth rate in the antagonist protocol (second groups 22.2 vs. 35.9% and third groups 23.9 vs. 41.5 %). Similarly, Orvieto & Patrizio [31] reported that live birth rates and ongoing pregnancy were significantly lower in the group treated with the GnRH antagonist when compared to the agonist long protocol. After analyzing nine trials and twenty eight RCTs for GnRH antagonist application in IVF, Youssef & Elashmawi [32] determined that, the live birth rate was 1.5% and 2% lower in the GnRH antagonist when compared to the agonist treatment. However, a recent review of Al-Inany et al. [29] that is contrary to their previous reports [1, 33], has demonstrated no evidence of statistically significant differences in the rates of live births or ongoing pregnancies when comparing GnRH agonist long

protocols with antagonist protocols. A meta-analysis by Xiao et al. [34] showed that ongoing pregnancy and live births were similar in the GnRH antagonist when compared with the standard long GnRH agonist protocols. Nonetheless, Conrad et al. [35] demonstrated significantly higher live birth rates in women provided with LH supplementation in antagonist cycles, where their intra-cycle LH levels were very low. The lower live birth rates in the antagonist protocol group in our study might be a result of a thinner endometrium and lower estradiol levels on HCG injection day when compared to the agonist protocol. It is well documented that inadequate estradiol levels from ovarian stimulation may impair endometrial receptivity [36,37]. Similarly, Orvieto et al.[38] showed a significantly lower endometrial thickness for the antagonist treatment when compared to the agonist. However, Simon et al.[39] demonstrated that endometrial development after GnRH antagonist mimics the natural endometrium to a greater extent than after GnRH agonist.

In our study, there was no difference in the clinical pregnancy between the second and third group of antagonist and agonist protocols as well between these two treatment protocols. Engel et al. [40] performed a sub-analysis of patients with equal demographic and clinical features, which resulted in similar pregnancy rates independent of GnRH used. Shanbhag et al.[41] and Orvieto et al.[38] observed a lower pregnancy rate in the antagonist protocol compared to the GnRH agonist long protocol. Ludwig et al.[42] in their meta-analysis study showed a reduction in the pregnancy rate using antagonist - ganirelix/ antagon vs. long agonist protocol. However, the antagonist cetrorelix resulted in the same pregnancy rate as with the long agonist protocol. The studies of Al-Inany & Aboulghar [43] and Depalo et al. [13] showed a trend towards a lower pregnancy rate in the antagonist protocol. A similar trend was also observed in patients with LH deficiency as documented by low E_2 to oocyte

ratio and was explained by the endometrial impact of a lower LH level [44]. Higher pregnancy rates in the agonist cycles may be a result of larger number of oocytes and embryos for selection for transfer [45]. Demonstrated differences in the number of M II and 2PNs between the studied groups of both protocols in our study were more likely to be a result of enforced stratification of the data. Therefore, in each group of both protocols, the number of mature and fertilized oocytes were progressively increasing with parallel increase in estradiol level in the blood produced by growing follicles. However, the overall number of oocytes retrieved, MII oocytes and 2PN oocytes in the antagonist protocol was significantly lower compared to the agonist in our study. Similarly, European and Middle East Study [46], Mochtar et al. [47], Barmat et al. [48] and Orvieto & Patrizio [31] reported a lower mean number of cumulus-oocyte-complexes (COC) and 2 pronuclear (PN) oocytes in the GnRH-antagonist group compared to the GnRH – agonist group. The reports of Albano et al. [10], Olivennes et al. [49], Fluker et al. [50], Roulier et al. [51] showed similar results to those obtained in our study, suggesting that they stem from a lower serum estradiol level on the day of hCG administration. A possible reason for a lower number of oocytes retrieved from the patients on the GnRH antagonist protocol compared to the long agonist protocol was suggested by Hurine et al.[52]. The authors claim that this is a result of a relatively higher level of FSH during early follicular phase that coincides with a range of initially developing follicles of the antagonist regiment, causing decreased synchronization of the follicular cohort [27] so that lower number oocytes were retrieved. Moreover, the differences between these two protocols might be due to a different mechanism of GnRH actions. In the agonist (long protocol) after a variable period of endogenous gonadotrophin depletion, small antral follicles are recruited by the exogenous gonadotropins. In contrast, in the antagonist cycle, the recruited follicles have

already been exposed for a few days to endogenous inter-cycle FSH rise [1,53,54]. Administration of the GnRH antagonist at the end of the stimulation period could have had an effect on the cell cycle of granulosa cells [55]. In vitro studies showed that GnRH-antagonist restrains cell growth by decreasing the synthesis and the stimulatory effects of IGFs on follicle growth [56]. GnRH may act as an autocrine factor by regulating mitogen-activated protein kinase in human granulosa luteal cells [57,58] and affect the follicles environment. Young et al. [59] demonstrated a difference in the follicular microenvironment between GnRH agonist long protocol and GnRH antagonist protocol. However, the authors were not able to show an effect of the follicular microenvironment on the clinical outcome (pregnancy and implantation rates), most likely due to a small number of analyzed cycles (n=32-antagonist and n=36-long GnRH agonist). The recent study on the morphokinetics of the embryos [17] demonstrated that abnormality in cleavage embryo (reverse cleavage) was associated with the regiment used for ovarian stimulation. Reverse cleavage was more frequently seen in the embryos where GnRH antagonists were used compared to GnRH agonists. The authors suggested that mechanisms controlling reverse cleavage may be sensitive to the environment of the oocyte during folliculogenesis. Therefore, it supports the earlier statement, that population of follicles and oocytes of long agonist and antagonist protocols differ from each other.

In conclusion, clinical outcomes appear to be influenced by the percentage of mature oocytes retrieved especially when the percentage of retrieved mature oocytes is low. It is essential to establish ovarian sensitivity to gonadotropins before any type of individualized approach of controlled ovarian stimulation protocol will be applied.

Reference

[1]   H. Al-Inany, M. Aboulghar. "GnRH antagonist for assisted conception". Cochrane *Database Syst. Rev,* 4, CD001750, 2001.

[2]   B. C. Fauser, K. Diedrich, P. Devroey. "Predictors of ovarian response: progress towards individualized treatment in ovulation induction and ovarian stimulation", Evian Annual Reproduction Workshop Group 2007. *Hum Reprod*, vol. 14, pp.1-14, 2008.

[3]   D. Bodri, S.K. Sunkara, A. Coomarasamy. "Gonadotropin-releasing hormone agonists versus antagonists for controlled ovarian hyperstimulation in oocyte donors: a systematic review and meta-analysis". *Fertil Steril*, vol. 95, pp.164-169, 2011.

[4]   S. Gordts, C. Van Turnhout, R. Campo, P. Puttemans, M. Valkenburg, S.A. Gordts, "Prospective randomized study comparing a GnRH-antagonist versus a GnRH-agonist short protocol for ovarian stimulation in patients referred for IVF". *FVV in Ob Gyn*, vol. 4, pp. 82-87, 2012.

[5]   E.G.Hughes, D.M. Fedorkow, S. Daya, M.A. Sagle, P. Van de Koppel, J.A. Collins. "The routine use of gonadotropin-releasing hormone agonists prior to in vitro fertilization and gamete intrafallopian transfer:

A metanalysis of randomized trials". *Fertil Steril*, vol. 58, pp. 888-896, 1992.

[6]   C. Albano, J. Smitz, M. Camus, H. Riethmuller-Wuller, M. Siebert-Weiger, K. Diedrich, et al. "Hormonal profile during the follicular phase in cycles stimulated with a combination of human menopausal gonadotrophin-releasing hormone antagonist". *Hum Reprod,* vol. 11, pp.14-18, 1996.

[7]   B.C. Fauser, P. Devroey. "Why is the clinical acceptance of gonadotropin-releasing hormone antagonist co-treatment during ovarian hyperstimulation for in vitro fertilization so slow?" *Fertil Steril,* vol. 83, pp.1607-1611, 2005.

[8]   G. Griesinger, C.A. Venetis, T. Marx, K. Diedrich, B.C. Tarlatzis, E.M. Kolibianakis. "Oral contraceptive pill pretreatment in ovarian stimulation with GnRH antagonists for IVF: a systematic review and meta-analysis". *Fertil Steril*, vol.90, pp.1055-1063, 2008.

[9]   Y.S. Choi, S.Y Ku, Ch.S. Suh, Y.M. Choi, J.G. Kim, S.Y. Moon, S.H. Kim. "Comparison of follicular fluid IGF-1, IGF-II, IGFBP-3, IGFBP-4 and PAPP-A concentrations and their ratios between GnRH agoinst and GnRH antagonist protocols for controlled ovarian stimulation in IVF-embryo transfer patients". *Hum Reprod*, vol. 21, pp.15-21, 2006.

[10] C. Albano, R.E. Felberbaum, J. Smitz, H. Riethmuller-Winzen, J. Engel, K. Diedrich et. al. "European Cetrorelix Study Group Ovarian stimulation with HMG: results of a prospective randomized phase III European study comparing the luteinizing hormone-releasing hormone (LHRH)-antagonist cetrorelix and the LHRH-agonist buserelin". *Hum Reprod,* vol. 15, pp. 526-531, 2000.

[11] M. R. Z. Rashid, F.B. Ong, M.H. Omar, S.P. Ng, A. Nurshaireen, N.S.M.N. Sharifah-The, A. H. Fazilah,

M.A. Zamzarina. "GnRH Agonist and GnRH Antagonist in Intracytoplasmic Injection Cycles". *Med J Malaysia*, vol. 63, pp. 113-117, 2008.

[12] B.K. Aygun, S. Kahraman . "Comparison of GnRH Agonist Long and Antagonist Protocols in the Same Normoresponders Patient Undergoing Assisted Reproductive Treatment". *Firat Med J*, vol. 15, pp. 123-127, 2010.

[13] R. Depalo, K. Jayakrishan, G. Garruti, I. Totaro, M. Panzarino, F. Giorgino, L.E. Selvaggi. "GnRH agonist versus GnRH antagonist in in vitro fertilization and embryo transfer (IVF/ET)". *Reprod Biol Endocrinol*, vol. 10, pp.1-8, 2012.

[14] J.T. Arruda, M.S. Approbato, M.C.S. Maia, T.M. Silva, C.R. Mendonca, M.S. Ramos. "Comparison GnRH agonist short protocol and GnRH antagonist in Brazilian normoresponder patients undergoing their first cycle of controlled ovarian stimulation". *JBRA Assist Reprod*, vol. 17, pp. 304-309, 2013.

[15] K.P. Mc Natty, S. D. Moore, A.J. Makris, et al. "The microenvironment of the human antral follicle: interrelationships among the steroid levels in antral fluid, the population of granulose cells and the status of oocytes in vivo and in vitro". *J Clin Endocrinol Metab*, vol. 49, pp. 851-860, 1979.

[16] T. Van Dessel, I. Schipper, D. Pache, et al. "Normal human follicle development: an evaluation of correlations with oestradiol, androstenedione and progesterone concentrations in individual follicles". *Clin. Endocrinol*, vol. 44, pp. 191-198, 1996.

[17] M. P. Teissier, H. Chable, S. Paulhac, Y. Aubard. "Comparison of follicle steroidogenesis from normal and polycystic ovaries in women undergoing IVF: relationship between steroid concentrations, follicle size, oocyte quality and fecundability". *Hum. Reprod*, vol. 15, pp. 2471-7, 2000.

[18] M. A. Santos, E.W. Kuijk, N. S. Macklon. "The impact of ovarian stimulation for IVF on the developing embryos". Reproduction, vol.139, pp. 23-34, 2010.

[19] Y. Liu, V. Chapple, M. R. Med, P. Roberts, P. Matson. "Prevalence, consequence, and significance of reverse cleavage by human embryos viewed with the use of the Embryoscope time-lapse video system". *Fert Steril*, vol. 102, pp.1295-300, 2014.

[20] L. Nilsson, M. Wikland, L. Hamberger, et. al. "Simplification of the method of in vitro fertilization: sonographic measurements of follicular diameter as the sole index of follicular maturity". *J In Vitro Fert Embryo Transf*, vol. 2, pp. 17-22, 1985.

[21] S. Simonetti, L.L. Veek, H.W. Jones Jr. "Correlation of follicular fluid volume with oocyte morphology from follicles stimulated by human menopausal gonadotropin". *Fertil. Steril*, vol. 44, pp. 177-190, 1985.

[22] R.T. Scott, G.E. Hofmann, S.J. Muasher, A.A. Acosta, D.K. Kreiner D K, Z. Rosenwaks. "Correlation of follicular diameter with oocyte recovery and maturity at time of transvaginal follicular aspiration". *J In Vitro Fert Embryo Transf*, vol. 6, pp. 73-75, 1989.

[23] L. Nayudu, D.A. Gook, A. Lopata, W.L.A Johnston. "Prediction of outcome in human in vitro fertilization based on follicular and stimulation response variables". *Fertil Steril*, vol. 51, pp. 117-125, 1989.

[24] C. Bergh, H. Broden, K. Lundin, L. Hamberger. "Comparison of fertilization, cleavage and pregnancy rates of oocytes from large and small follicles". *Hum Reprod*, vol. 13, pp. 1912-1915, 1998.

[25] J. M. Knopman, J. A. Grifo, A. P. Novetsky, M.B. Smith, A.S. Berkeley. "Is bigger better: The association between follicle size and live birth rate following IVF?" *J Obstet. Gynaecol*, vol. 2, pp. 361-366, 2012.

[26] K. F. Miller, J. M. Goldberg, T. Falcone." Follicle size and implantation of embryos from in vitro fertilization". *Obstet Gynecol*, vol. 88, pp. 583-586, 1996.

[27] D. Nogueira, S. Friedler, M. Schachter, A. Raziel, R. Ron-El, J. Smitz. "Oocyte maturity and preimplantation development in relation to follicular diameter in gonadotropin-releasing hormone agonist or antagonist treatment". *Fertil Steril*, vol. 85, pp.578-583, 2006.

[28] M. P. Rosen, S. Shen, A.T. Dobson, P. F. Rinaudo, C.E. McCulloch, M. I. Cedars. "A quantitative assessment of follicle size on oocyte developmental competence". *Fertil Steril,* vol. 90, pp. 684-690, 2008.

[29] H. Y. Al-Inany, M. A. F. M Youssef, M. Aboulghar, F. J. Broekmans, M.D. Sterrenburg, J.G. Smith, A.M. Abou-Setta. "Gonadotrophin -releasing hormone antagonists for assisted reproductive technology". *Cochrane Database Syst Rev*, 5, CD001750, 2011.

[30] J. Xiao, S. Chen, C. Zhang, S. Chang. "Effectiveness of GnRH antagonist in the treatment of patients with polycystic ovary syndrome undergoing IVF: a systematic review and meta analysis". *Gynecol Endocrinol*, vol. 29, pp. 187-91, 2013.

[31] R. Orvieto, P. Patrizio. "GnRH agonist versus GnRH antagonist in ovarian stimulation: an ongoing debate". *Reprod Biomed Online*, vol. 26, pp. 4-8, 2013.

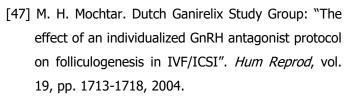[32] M. A. F.M. Youssef, H. Elashmawi. "GnRH antagonist for pituitary desensitization in IVF: Is it a time for a change of practice?" *Middle East Fertility Society Journal*, vol. 16, pp. 254-258, 2011.

[33] H. Al-Inany, A. M. Abou-Setta, M. Aboughar. "Gonadotropin -releasing hormone antagonists for assisted reproductive technology". *Cochrane Database Syst. Rev*, 3, CD001750, 2006.

[34] J. S. Xiao, C. M. Su, X. T. Zeng. "Comparison of GnRH Antagonist versus GnRH Agonist Protocol in Supposed Normal Ovarian Responders Undergoing IVF: A Systematic Review and Meta-Analysis". *PLoS One,* vol. 12, 9(9), 2014; e 106854. Doi: 10.1371/journal.pone.0106854.eCollection 2014.

[35] D. Conrad, G. Hughes, G. Sacks. "LH supplementation for low LH levels during antagonist IVF cycles improves live birth rates". *JFiV Reprod Med Genet*, vol. 3, no. 155, 2015. doi: 10.4172/2375-4508.1000155.

[36] A. Pellicer, D. Valbuena, F. Cano, J. Remohi, C. Simon. "Lower implantation rates in high responders: evidence for an altered endocrine milieu during the preimplantation period". *Fertil Steril*, vol. 65, pp.1190-5, 1996.

[37] R. J. Paulson, M. V. Sauer, R. A. Lobo. "Embryo implantation after human in vitro fertilization: importance of endometrial receptivity". Fertil Steril, vol. 53, pp. 870-874, 1990.

[38] R. Orvieto, S. Meltcer, J. Rabinson, E. Zohav, E. Y. Anteby, R. Nahum. "GnRH agonist versus GnRH antagonist in ovarian stimulation: the role of endometrial receptivity?" *Fertil Steril*, vol. 90, pp. 1294-1296, 2008.

[39] C. Simon, J. Oberye, C. Vidal, E. Bosch, J.A Horcajadas, C. Murphy, S. Adams, A. Riesewijk, B. Mannaerts, A Pellicer. "Similar endometrial development in oocyte donors treated with either high-or standard-dose GnRH antagonist compared
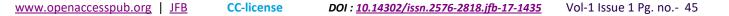
to treatment with a GnRH agonist or in natural cycles". *Hum Reprod*, vol. 20, pp. 318-327, 2005.

[40] J. B. Engel, G. Griesinger, A. Schultze-Mosgau, R. Felberbaum, K. Diedrich. "GnRH agonists and antagonists in assisted reproduction pregnancy rate". *Reprod Biomed Online*, vol. 13, pp. 84-87, 2006.

[41] S. Shanbhag, L. Aucott, S. Bhattacharya, M.A. Hamilton, A.R. McTavish. "Intervention for poor responders to controlled ovarian hyperstimulation (COH) in in-vitro fertilization (IVF)". *Cochrane Database Syst Rev*, 1, CD004379, 2007.

[42] M. Ludwig, A. Katalinic, K. Diedrich. "Use of GnRH antagonists in ovarian stimulation for assisted reproductive technologies compared to the long protocol. Meta-analysis". *Arch Gynecol Obstet*, vol. 265, pp. 175-182, 2001.

[43] H. Y. Al-Inany, M. Aboughar. "GnRH antagonist in assisted reproduction: a Cochrane review". *Hum Reprod*, vol. 17, pp.874-885, 2002.

[44] I. Cedrin-Durnerin, D. Grange-Dujardin, A. Laffy, I. Parneix, N. Massin, J. Galey, L. Theron, J. P. Wolf, C. Conord, P. Clement, S. Jayot, J.N. Huges. "Recombinant human LH supplementation during GnRH antagonist administration in IVF/ICSI cycles; a prospective randomized study". *Hum Reprod*, vol. 19, pp. 1979-1984, 2004.

[45] C Hayden. "GnRH analogues: applications in assisted reproductive techniques". *European Journal of Endocrinology*, vol. 159, S17-25, 2008.

[46] European and Middle East Orgalutan Study Group. "Comparable clinical outcome using the GnRH antagonist ganirelix or a long protocol of the GnRH agonist triptorelin for the prevention of premature LH surges in women undergoing ovarian stimulation". *Hum Reprod*, vol. 16, pp. 644-651, 2001.

[47] M. H. Mochtar. Dutch Ganirelix Study Group: "The effect of an individualized GnRH antagonist protocol on folliculogenesis in IVF/ICSI". *Hum Reprod*, vol. 19, pp. 1713-1718, 2004.

[48] L. I. Barmat, S. J. Chantilis, B. S. Hurst, R. P.A. Dickey. "A randomized prospective trial comparing gonadotropin releasing hormone (GnRH) antagonist/recombinant follicle-stimulating hormone (rFSH) versus GnRH-agonist/rFSH in women pretreated with oral contraceptives before in vitro fertilization". *Fertil Steril*, vol. 83, pp. 321-330, 2005.

[49] F. Olivennes, J. Belaisch-Allart, J. C. Empereraire, H. Dechaud, S. Alvarez, L. Moreau, et al. "Prospective, randomized, controlled study of in vitro fertilization-embryo transfer with a single dose of a luteinizing hormone-releasing homone (LHRH) antagonist (cetrorelix) or a depot formula of an LHRH agonist (triptorelin)". *Fertil Steril*, vol. 73, pp. 314-320, 2000.

[50] M. Fluker, J. Grifo, A. Leader, M. Levy, D Meldrum, S. J. Muasher, J. Rinehart, Z. Rosenwaks, R. T. Scott Jr, W. Schoolcraft, et al. "Efficacy and safety of ganirelix acetate versus leuproide acetate in women undergoing controlled ovarian hyperstimulation". *Fertil Steril,* vol. 75, pp. 38-45, 2001.

[51] R. Roulier, V. Chabert-Orsini, M. C. Sitri, B. Barry, P. Terriou. "Depot GnRH agonist versus the single dose GnRH antagonist regiment (cetrolelix, 3 mg) in patients undergoing assisted reproduction treatment". *Reprod BioMed Online*, vol. 7, pp. 185-189, 2003.

[52] J. A. Huirne, R. Homburg, C. B. Lambalk. "Are GnRH antagonists comparable to agonists for use in IVF?" *Hum Reprod*, vol. 22, pp. 2805-2813, 2007.

[53] K. Elter, L.R. Nelson. "Use of third generation gonadotropin releasing hormone antagonists in in

vitro fertilization-embryo transfer: a review". *Obstet Gynecol Surv*, vol. 56, pp. 576-578, 2001.

[54] F. Olivennes, J.S. Cunha-Filho, R. Fanchin, P. Buchard, R. Frydman. "The use of GnRH antagonists in ovarian stimulation". *Hum Reprod Update*, vol. 8, pp. 279-290, 2002.

[55] E. R. Hernandez. "Embryo implantation and GnRH antagonists: embryo implantation: the Rubicon for GnRH antagonists". *Hum Reprod*, vol. 15, pp. 1211-1216, 2000.

[56] G. Emons, O. Ortmann, M. Becker, G. Irmer, B. Springer, R. Laun, F. Holzel, K.D. Schulz, A. V. Schally. "High affinity binding and direct antiproliferative effects of LHRH analogues in human ovarian cancer cell lines". *Cancer Res*, vol. 15, pp. 5439-5446, 1993.

[57] S. K. Kang, K. C. Choi, K. W. Cheng, P.S. Nathwani, N. Auersperg, P.C. Leung. "Role of gonadotropin-releasing hormone as an autocrine growth factor in human ovarian surface epithelium". *Endocrinol*, vol. 141, pp. 72-80, 2000.

[58] S. K. Kang, K. C. Choi, C. J. Tai, N. Auersperg, P.C. Leung. "Estradiol regulates gonadotropin-releasing hormone (GnRH) and its receptor gene expression and antagonizes the growth inhibitory effects of GnRH in human ovarian surface epithelium and ovarian cancer cells". *Endocrinol*, vol. 142, pp. 580-588, 2001.

[59] S. CH. Young, K. Seung-Yup, J. Byung-Chul, S. Chang-Suk, M. CH. Young, G. K. Jung, Y. M. Shin, H.K. Seok. "Comparison of follicular fluid IGF-I, IGF-II, IGFBG-3, IGFBP-4 and PAPP-A concentrations and their ratios between GnRH agonist and GnRH antagonist protocols for controlled ovarian stimulation in IVF-embryo transfer patients". *Hum Reprod*, vol. 21, pp. 2015-2021, 2006.