Introduction

The follicular response to controlled ovarian hyperstimulation (COH) with Follicular Stimulating hormone (FSH) or Human Menopausal Gonadotropin (HMG) is essential to the performance of assisted reproduction by in-vitro fertilization/intra-cytoplasmic sperm injection (IVF/ ICSI). The possibility to fertilize more than one oocyte clearly improves the chances for a successful treatment compared with the limited prognosis after a natural unstimulated cycle ***(Stoop et al., 2012).***

Failure to respond adequately to standard protocols and to recruit adequate follicles is called "poor ovarian response". This results in decreased oocyte production, cycle cancellation and, overall it is associated with a significantly diminished probability of pregnancy ***(Venetis et al., 2010).***

The occurrence of poor response to ovarian stimulation is not infrequent; the prevalence of poor responders is 12 % ***(Klinkert et al., 2004).***

However, there is still no universal definition of the response groups although the terms are widely used in research and in daily clinical practice. The inconsistency in the definition of the poor-response group has been the subject of many studies.

In this study poor ovarian response (POR) will be determined as having one of the following criteria in the current cycle:

- Low serum Estradiol (E2) levels (500 pg/ml or less) measured on the day of Human Chorionic Gonadotropin (HCG) administration ***(Davar et al., 2011).***

Or

- Less than four oocytes retrieved on the day of ovum pick up ***(Ozmen et al., 2009).***

The etiology of poor response to ovarian stimulation is unknown. Despite being highly correlated with maternal age, the condition is also common in younger women in whom low ovarian reserve represents the most frequent etiological factor ***(Mahutte et al., 2002).***

In addition, low ovarian reserve may be associated with advanced endometriosis, prior ovarian surgery, pelvic adhesions, increased body mass index and smoking ***(Nargund et al., 1995).***

However, poor response to ovarian stimulation might also occur unexpectedly in young women who are non-smoker and have apparently normal ovarian reserves ***(Nikolaou et al., 2003).***

Studies have shown that poor ovarian response is the first sign of ovarian aging (early ovarian failure or early menopause). This is clinically displayed by a shortened follicular phase which limits the time available to recruit an adequate number of follicles.

Suggested mechanisms for poor ovarian response include decreased number of Follicular Stimulating hormone (FSH) receptors in granulosa cells, defective signal transduction after Follicular Stimulating hormone (FSH) receptor binding, inappropriate local vascular network for the distribution of gonadotropins, presence of autoantibodies against granulosa cells, an excess of vascular endothelial growth factor receptor (VEGFR-1), abnormality in Insulin like growth factor-I (IGF-I) and Insulin like growth factor- II (IGF-II) levels and diminished circulating gonadotropin surge-attenuating factor bioactivity (GnSAF) ***(Ulug et al., 2007).***

Immunological abnormalities have been implicated in female reproductive failure, but whether these represent a cause or effect is unknown ***(Sarapik et al., 2010).***

Cytokines, originally identified as products of immune cells, are important mediators of immune responses. These proteins are able to stimulate or inhibit cell growth, regulate cell differentiation, induce cell chemotaxis and modulate the expression of other cytokines. However, recent research has indicated that cytokines are synthesized by a broad range of non-immune cell types, including the normal ovarian cells. Cytokine function in the ovary has been described as promoting processes of follicular growth, steroidogenesis, recruitment and activation of leukocytes necessary for ovulation and tissue remodelling during ovulation, luteinization and luteolysis ***(Buscher et al., 1999).***

Interleukin 6 (IL-6) was first identified as a T-cell derived cytokine that promotes B-cell differentiation and antibody production. Many studies since have shown IL-6 to be a pleiotrophic cytokine with multiple cellular effects ranging from growth promotion, growth inhibition and cell differentiation to inflammation, hematopoiesis, neuronal function and osteoclastogenesis. Pathogenic implications of IL-6 are described for several diseases ranging from autoimmune disorders to malignancies ***(Kishimoto et al., 2005).***

Interleukin 6 (IL-6) expression is described within the human granulosa cells of the Graafian follicle, in the human corpus luteum and ovarian theca cells, the endometrium and in the pre-implantation embryo. Measurements of follicular fluid (FF) levels of IL-6 in women undergoing in vitro fertilization (IVF) have been shown previously to be associated with etiology of infertility, stimulation protocol, fertilization rates and pregnancy outcome ***(Richards et al., 2008).***

However, the mechanistic link between elevated levels of Interleukin 6 (IL-6) and poor oocyte quality is still unclear. The direct role of this cytokine as a possible mediator for impaired oocyte spindle and chromosomal structure has been studied by ***Banerjee et al. (2012)***, in which metaphase-II mouse oocytes were exposed to recombinant mouse IL-6 and subjected to indirect immunofluorescent staining to identify alterations in the microtubule and chromosomal alignment compared to untreated controls. Results of this study showed that IL-6 caused a dose-dependent deterioration in microtubule and chromosomal alignment in the treated oocytes as compared to the untreated group, giving a conclusion that elevated levels of IL-6 associated with endometriosis and pelvic inflammation may reduce the fertilizing capacity of human oocyte through a mechanism that involves impairment of the microtubule and chromosomal structure ***(Banerjee et al., 2012)***.

Aim of the Work

This study aims to find a correlation between level of follicular fluid Interleukin 6 (IL-6) on the day of oocyte retrieval and poor ovarian response after controlled ovarian hyperstimulation in infertile women undergoing in-vitro fertilization/ intra-cytoplasmic sperm injection (IVF/ICSI) cycle.

Chapter (1)
Poor responders

In the field of assisted reproductive technologies great steps forward have been made in recent years in terms of clinical knowledge and technological development especially in IVF laboratories. One of the fundamental steps to reach the success is still related to the number of eggs obtained after hormonal stimulation by gonadotropins in combination with GnRH analogues.

In patients defined as poor responders, the limited number of obtained eggs remains the main problem in optimizing the live birth rates. In fact, as a result of a lower number of oocytes retrieved, there are fewer embryos to select and transfer and subsequently these patients have lower pregnancy rates per transfer and lower cumulative pregnancy rates per started cycle compared with normal responders.

Definitions & Incidence of Poor Ovarian Responders:

Although the concept of poor ovarian response was introduced over 30 years ago, we had not had a common definition of poor responder patients. In fact, ***Polyzos et al. (2011)*** emphasized enormous variability of the definitions of poor responder patients proposals from the literature. These results confirm the difficulty in obtaining an exact incidence of this condition (that has been estimated at 9–24% but it seems to be slightly increased in the last decade), the incapacity to compare the results of different trials and therefore to identify the best treatment.

A huge variety of definitions for poor ovarian response have been proposed and published in the literature:

- The number of mature follicles on the day of human chorionic gonadotropin (HCG) administration <2 to <5 ***(Land et al., 1996, Surrey et al., 1998 and Lashen et al., 1999).***
- Number of oocytes retrieved <4 to <6 ***(Faber et al., 1998 and Rombauts et al., 1998).***
- Serum estradiol concentrations <100 pg/mL on day 5 of stimulation or <300 to <600 pg/mL on the day of HCG ***(Lindheim et al., 1996, Schoolcraft et al., 1997 and Surrey et al., 1998).***
- Total gonadotropin dose used or the daily stimulation dose ***(Hughes et al., 1994, Ibrahim et al., 1997 and Vishvanath et al., 1997).***
- Prolonged duration of gonadotropin stimulation ***(Toth et al., 1997).***

Recently in 2011, the ESHRE working group on poor ovarian response has finally given a common definition of poor responder named **"The Bologna Criteria"**, where at least two of the following three features must be present:

(a) Advanced maternal age or any other risk factor for poor ovarian response (POR)
(b) A previous POR
(c) An abnormal ovarian reserve test.

Ferraretti et al., (2011)

The Bologna Criteria:

In 2011, a panel of experts in reproductive medicine gathered together in an ESHRE Campus on poor responders held in Bologna, Italy with the aim to find a common and

universal definition of poor ovarian response trying to find simple, clearly defined and reproducible criteria.

The Bologna ESHRE criteria represent the first real attempt by the scientific community to unify the many definitions proposed to identify poor responder patients by establishing a definite point from which to begin and how to find therapeutic strategies.

It was concluded that poor ovarian responders should be considered patients having at least two of the following criteria:

- A previous episode of poor ovarian response (≤3 oocytes) with a standard dose of medication
- An abnormal ovarian reserve with AFC <5–7 follicles or AMH <0.5–1.1 ng/mL
- Women above 40 years of age or presenting other risk factors for poor response such as previous ovarian surgery, genetic defects, chemotherapy, radiotherapy, and autoimmune disorders.

Ferraretti et al. (2011)

The "Bologna criteria" although accepted by the vast majority of the scientific community have raised some criticism. A letter to the editor on the ESHRE consensus on the definition of poor ovarian response to ovarian stimulation ***(Younis et al., 2012)*** underlines that although the first step has been made with the introduction of "Bologna criteria", "the work is yet to be accomplished." In accordance with the literature, guidelines for physicians should be necessary to identify risk factors and to integrate these with the diagnosis of poor ovarian response, especially for young women.

Faddy et al. (1992) observed a physiological decline of the follicular heritage in every woman over time with a marked increase in the rate of follicular disappearance from

age 37 to 38 years onwards. From this moment, the time to the menopause takes about 10–13 years. In poor responders the mechanism of ovarian insufficiency is prematurely determined and not fully understood. Some causes of decrease in ovarian reserve have been identified:

- Ovarian surgery especially in case of endometrioma ***(Benaglia at al., 2010, De Ziegler et al., 2010, Raffi et al., 2012 and Streuli et al., 2012)***.

- Genetic defects.
- Chemotherapy.
- Radiotherapy.
- Autoimmune disorders.
- Single ovary.
- Chronic smoking.
- Unexplained infertility.

(De Vos et al., 2010)

Moreover, new risk factors of low ovarian response have been proposed:

- Diabetes mellitus Type I ***(Soto et al., 2009).***
- Transfusion-dependent B-thalassemia ***(Chang et al., 2011).***
- Uterine artery embolization for the treatment of uterine leiomyoma ***(Hehenkamp et al., 2007 and Tropeano et al., 2010).***

However, in most cases the mechanism involved in follicular depletion is still not clear ***(De Vos et al., 2010)***.

Other etiologies rather than diminished ovarian reserve have been suggested as:

- Decreased number of FSH receptors available in granulosa cells ***(Zeleznik et al., 1981)***.

- Defective signal transduction after FSH receptor binding ***(Hernandez et al., 1992)***.
- The presence of a special FSH receptor-binding inhibitor in the follicular fluid ***(Lee et al., 1993)***.
- An inappropriate local vascular network for the distribution of gonadotrophins ***(Pellicer et al., 1994)***.
- The presence of autoantibodies against granulosa cells ***(Pellicer et al., 1998)***.
- Lowered circulating gonadotrophin surge-attenuating factor (GnSAF) bioactivity ***(Martinez et al., 2002)***.

It has been suggested that the reduced number of oocytes could be correlated to a reduction of their quality, which is clinically translated into a reduction of the implantation rates and an increase of early pregnancy loss ***(Liu et al., 2011)***. Conversely, because of the lack of a clear correlation between quantity and quality, different authors have suggested that poor responders do not possess a lower chance of success in IVF, with the age of the woman being the most important predictor of live birth rate ***(Ulug et al., 2003 and Zhen et al., 2008).***

However, very large studies have shown that this group of patients has reduced pregnancy rates compared with normal responders independently from the treatment protocol ***(Sunkara et al., 2007)*** used and from the age of the patient ***(Toukhy et al., 2002 and Polyzos et al., 2014)***. In this group of difficult patients it is thus clear that to optimize the clinical results in IVF, it is not only important to predict the ovarian reserve but also to tailor the best stimulation protocol to exploit fully the ovarian reserve and optimize the number of oocytes to be retrieved. Only with recent advent of new reliable biomarkers of ovarian reserve better strategies for the management of these patients have been suggested ***(Al-Azemi et al., 2011)***.

Why this topic is so controversial?

The difficulties encountered by researchers in finding real options in the treatment of poor responders are due to the following:

- Many studies are represented by a small number of patients limiting the power to find a significant difference between the various treatments.
- Numerous definitions of poor responders reported in the literature have led to the presence of a heterogeneous group of patients.
- Causes and mechanisms leading to poor ovarian reserve are still unclear, especially in young women, they are largely unknown.
- Different end points have been used in the studies to evaluate the outcomes of this group of patients.
- Impossibility to compare results from different studies due to the presence of numerous bias.
- Limited value of some meta-analyses ruling out many observational studies.

In terms of what we discussed, reviews and meta-analyses conclude that there is insufficient evidence to recommend the use of particular intervention to improve outcomes in this group of patients.

(Ubaldi et al., 2014)

Management of poor responder group:

Although many protocols with different doses and types of gonadotropins have been proposed in the literature over the past 20 years for the management of poor responder patients, to date there is no really efficient treatment that could solve the problem of poor ovarian response and the current question is still; which is the ideal protocol for patients defined as poor responders?

- **Gonadotropins:** When the standard dose of gonadotropins (225–300 IU) fails to induce a proper multifollicular growth, the obvious clinical approach is to increase the dose. High doses of gonadotropins have been thus used for a couple of decades by the vast majority of the authors in poor responder patients. In the literature conflicting data are however reported on the outcomes of this approach: some ***(Karande et al., 1990, Van Hooff et al., 1993 and Land et al., 1996)*** but not all authors ***(Hofmann et al., 1989)*** in prospective and retrospective studies did not report enhanced ovarian response and/or better pregnancy rates when the starting dose of gonadotropins was increased up to 450 IU. More recently ***Berkkanoglu et al., (2010)*** confirmed that the increase of FSH starting dose does not result in higher pregnancy rates and also found no differences between the starting dose of 300 IU, 450 IU, and 600 IU of gonadotropins in terms of retrieved oocytes, number of embryos obtained, and pregnancy rates. It is today clear that these patients have a reduced ovarian reserve; the recruitable follicles are fewer and the gonadotropins, independently of the dosage administered, can only support the cohort of follicles receptive to stimulation without manufacturing follicles de novo.

- **GnRH Analogues:** From the beginning of the nineties the combination of gonadotropins and gonadotropin-releasing hormone (GnRH) agonists, started on the late luteal phase of the previous cycle, has been considered the protocol of choice in good responder patients. This approach lowers cancellation rate and raises the number of preovulatory follicles and the number of oocytes retrieved and good quality embryos for transfer, leading to better pregnancy rates ***(Hughes et al., 1992)***. However this protocol could have a detrimental effect in poor responders because it may induce an excessive ovarian suppression that could

lead to a reduced or absent follicular response ***(Yoshimura et al., 1992 and Kowalik et al., 1998)***. For this reason, in patients with poor ovarian reserve the options could be:

i. To decrease the length of suppression by decreasing the duration of GnRH agonist use (short and ultra-short, mini- and microdose flare up regimens) ***(Padilla et al., 1996, Schoolcraft et al., 1997 and Surrey et al., 1998).***
ii. To lower or to stop (after pituitary suppression) the dose of GnRH agonists initiated during the luteal phase ***(Faber et al., 1998 and Karande et al., 1999).***
iii. To use the GnRH antagonists in combination with gonadotropins to prevent premature LH rise during the mid-late follicular phase ***(Craft et ., 1999 and Akman et al., 2000)***.

- **GnRH Antagonist:** The use of GnRH antagonist was introduced in the clinical practice about 15 years ago. The most important advantages of the use of GnRH antagonist in combination with gonadotropins are improvement of patient's compliance, decreased number of days of stimulation and of the amount of gonadotropin administered and statistically significant reduction of ovarian hyperstimulation syndrome (OHSS). Furthermore, GnRH antagonists are not administered during the stage of follicular recruitment and thus suppression of endogenous gonadotropins secretion is not present at that time in contrast to GnRH agonists being a possible advantage during ovarian stimulation in this group of patients. For these reasons, several authors suggested the use of GnRH antagonists in combination with gonadotropins as a suitable protocol for poor responders. In fact, GnRH antagonists in the mid-late follicular phase during ovarian stimulation prevent the premature LH surge while not causing suppression in the early follicular phase, obtaining

more natural follicular recruitment without any inhibitory effect possibly induced by the GnRHagonist ***(Kenigsberg et al., 1984)***.

- **Alternative Approaches:** Several alternative approaches have been proposed with the aim of strengthening the effect of exogenous gonadotropins:

i. *Addition of Estradiol in the luteal phase* decreases the risk of cycle cancellation and increases the chance of clinical pregnancy in poor responder patients ***(Reynolds et al., 2013)***. The biological rationale might be that luteal estradiol priming could improve synchronization of the pool of follicles available to controlled ovarian stimulation ***(Fanchin et al., 2003)***.

ii. *Addition of Recombinant LH.* Some authors suggested the addition of recombinant LH during gonadotropin stimulation in poor responder patients ***(Hill et al., 2012)***.

iii. *Addition of Growth Hormone.* It has been suggested that the use of growth hormone (GH) might modulate the action of FSH on granulosa cells by upregulating the local synthesis of insulin-like growth factor-I (IGF-I) ***(Davoren et al., 1986, Hsu et al., 1987 and Barreca et al., 1993)***. The IGF-I amplifies the effect of FSH at the level of both granulosa and theca cells ***(Adashi et al., 1985 and Jia et al., 1986)***.

iv. *Addition of Androgens (DHEA).* Androgens, produced primarily by theca cells, play a critical role for an adequate follicular steroidogenesis ***(Ryan et al., 1968)*** and for a correct early follicular and granulose cell development ***(Weil et al., 1998)***. They are the substrate for the aromatase activity of the granulosa cells, which

converts the androgens to estrogens. Moreover, androgens may increase FSH receptor expression in granulosa cells amplifying the effects of FSH and thus potentially enhance responsiveness of ovaries to FSH ***(Vendola et al., 1998, Weil et al., 1998 and Kolibianakis et al., 2009)***. Furthermore, inadequate levels of endogenous androgens are associated with decreased ovarian sensitivity to FSH and low pregnancy rates after IVF ***(Frattarelli et al., 2004 and Kolibianakis et al., 2009)***.

v. *Addition of Aspirin and Heaprin.* Increased intra ovarian vascularity has been linked to improved delivery of gonadotropic hormones or other growth factors required for folliculogenesis ***(Weiner et al., 1993 and Bassil et al., 1997).*** On the other hand, impaired ovarian blood flow could contribute to poor ovarian response ***(Pellicer et al., 1994 and Battaglia et al., 2000)***. Based on this rationale, by enhancing ovarian vascularization with vasoactive substances such as aspirin, the ovarian response could theoretically improve ***(Rubinstein et al., 1999)***. However, the evidence supporting the effect of a low dose of aspirin in women undergoing IVF is poor and controversial ***(Nardo et al., 2009).***

vi. *Adjunctive use of glucocorticosteroids (dexamethasone).* It has been suggested that dexamethasone may directly influence follicular development and oocyte maturation via its isoform (11-B HSD) in the granulosa cells ***(Smith et al., 2000)*** or indirectly, by increasing serum GH and consequently intrafollicular IGF-1 ***(Miell et al., 1993)***. In addition, it may provoke immunosuppression within the endometrial microenvironment ***(Polak de Fried et al., 1993)***.