

## Clomiphene Citrate and Oocyte Quality



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### Abstract

The clomiphene citrate (CC) is a first line of medicine used for ovulation induction in women worldwide. CC has good ovulation induction ability in anovulatory women but the pregnancy rate is very poor. This discrepancy might be due to the anti-estrogenic effect of CC at various level including ovary and oocytes. The hypoestrogenic conditions due to CC treatment inhibit follicular growth and development, induce susceptibility of oocytes towards apoptosis and deteriorate oocyte quality after ovulation. CC induces reactive oxygen species (ROS) mediated granulosa cells as well as oocyte apoptosis within the follicle of the ovary. Apoptosis deteriorates oocyte quality and thereby reproductive outcome. Supplementation of estradiol 17 $\beta$  or natural antioxidant such as melatonin prevents anti-estrogenic effects of CC and improves oocyte quality by scavenging CC induced generation of ROS. Thus, we propose that the supplementation of estradiol 17 $\beta$  and/or melatonin along with CC may be beneficial to overcome the anti-estrogenic effect of CC during infertility management in human.

**Keywords:** Clomiphene citrate; Ovulation induction; ROS; Apoptosis; Oocyte quality

**List of Abbreviations:** ROS: Reactive Oxygen Species; CC: Clomiphene Citrate; ER: Estrogen Receptors; GnRH: Gonadotropin-Releasing Hormone; M-II: Metaphase-II; H<sub>2</sub>O<sub>2</sub>: Hydrogen Peroxide; ART: Assisted Reproductive Technology

### Introduction

Ovary is a dynamic organ and generates excess amount of reactive oxygen species (ROS) during follicular growth, development, maturation and ovulation [1]. The increased level of ROS is scavenged by antioxidant systems [2]. A moderate increase of ROS could be beneficial for oocyte meiotic maturation and reproductive outcome [3-5]. Overproduction of ROS or depletion of enzymatic antioxidant systems causes oxidative stress [2].

Anovulation is one of the major causes of reproductive failure in sub-fertile and infertile women [6,7]. In the absence of other reproductive problems, successful ovulation induction and good quality oocyte often restores normal fertility in human [8]. In a common clinical practice, gonadotropins are used to stimulate ovary in infertile or subfertile women [9,10]. However, anti-estrogens are widely used to induce ovulation in these patients. Clomiphene citrate (CC) is a non-steroidal ovulation-inducing drug that has been used in humans for more than 40 years [11,12]. Ovarian stimulation by gonadotropins is an expensive

treatment. CC is an inexpensive, safe and effective alternative to gonadotropins [11].

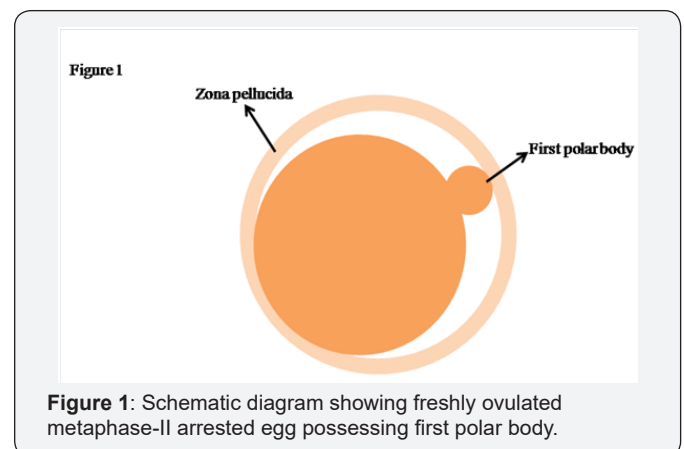


Figure 1: Schematic diagram showing freshly ovulated metaphase-II arrested egg possessing first polar body.

For ovulation induction, CC has been used as a first line of medicine [12]. It is similar to estrogen and thus binds to estrogen receptors (ER) [13]. It triggers hypothalamic gonadotropin-

releasing hormone (GnRH) secretion that stimulates release of pituitary gonadotropins [14]. Pituitary gonadotropins surge trigger growth and development of ovarian follicles [13,15]. CC treatment generates dominant follicle and results in the ovulation of metaphase-II (M-II) arrested oocytes required for successful fertilization in various assisted reproductive technology (ART) programs (Figure 1). Although CC has been used for ovulation induction widely, the possible mechanism of its action at the level of ovary still remains ill understood.

In spite of having good ovulation induction ability of CC (60% - 85%), the pregnancy rate is much lower (10% -20%) [10,16]. The higher incidence of miscarriage has been reported in the conception cycle after CC treatment [17]. Such a discrepancy could be due to anti-estrogenic effect of CC, particularly at the level of ovary, cervical mucus and endometrium [17,18]. The anti-estrogenic effects of CC may result apoptosis in granulosa cells and oocyte in ovary [6]. Studies suggest that CC induces apoptosis in human granulosa cells cultured *in vitro* [19]. CC induces granulosa cell apoptosis that reduces estradiol 17 $\beta$  level in the ovary of rat [20, 21] as well as in monkey [22].

The CC induced hypoestrogenic conditions may induce generation of ROS [6]. Animal studies suggest that CC treatment increases hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) level and reduces catalase activity in rat [21]. The increased level of ROS induces overexpression of bax protein and thereby DNA fragmentation both in granulosa cells and oocytes [20,21,24-32]. Granulosa cell apoptosis results in reduced estradiol 17 $\beta$  level in ovary [21,24-26]. The reduced level of estradiol 17 $\beta$  may deteriorate oocyte quality by inducing susceptibility of oocytes towards apoptosis. These oocytes are not of good quality and their use reduces ART outcome.

The anti-estrogenic condition due to CC treatment is one of the major side effects that affects the development and maturation of oocytes in the ovary and reduces oocyte quality after ovulation. To overcome the anti-estrogenic effect of CC, supplementation of exogenous estradiol 17 $\beta$  has been recommended [20,23, 33]. This is further supported by the observations that the exogenous estradiol 17 $\beta$  prevents apoptosis and thereby deterioration of oocyte quality [20,23].

The antiestrogenic effect of CC can also be prevented by the supplementation of a naturally occurring antioxidant such as melatonin [21]. Melatonin scavenges free radicals [34], induces superoxide dismutase [35] and glutathione peroxidase activities to prevent oxidative damage in human [36]. Animal studies suggest that melatonin induces catalase activity, reduces H<sub>2</sub>O<sub>2</sub> level and prevents CC-induced oocyte apoptosis [21].

## Conclusion

Based on existing clinical as well as animal studies, we conclude that use of either estradiol 17 $\beta$  or melatonin along with CC would be beneficial to overcome the anti-estrogenic effects of CC at the level of ovary during infertility management in human.

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## Conflict of Interest

The authors declare that no conflict of interest exists.

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