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Short Communication

Plasma Interleukin-1β (IL-1β) Concentrations at the Different Phases of the Oestrus Cycle in Clomiphene Treated Female Rats

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Summary: Interleukin - 1β (IL-1β) is a signal molecule known for its role in inflammation and immune response. However, there are reports on the possible effects of IL-1β in ovulation and various aspects on female reproduction. It was therefore necessary to determine plasma IL-1β levels in female rats administered ovulation inducing agent clomiphene citrate. In this work, a total of forty (40) female wistar rats weighing between 150g and 225g were used. Twenty of the rats were administered 0.14mg/kg clomiphene citrate orally daily for five days while the other twenty which served as control, received normal saline. The phases of the oestrus cycle (proestrus, estrus, metestrus and diestrus) were determined between the hours of 8.30am and 10.00am on the sixth day prior to collection of blood sample by cardiac puncture. The plasma IL-1β concentrations were determined using rat IL-1β ELISA kits. From the experiment, 41.2% of the control rats were in the diestrus phase while 42.1% of the clomiphene citrate treated rats were in the estrus phase. The IL-1β plasma concentrations were higher in clomiphene treated rats at all the phases of the oestrus cycle of experimental rats when compared with the control. The increase in plasma IL-1β was significant ($p<0.05$) in the estrus phase of the clomiphene citrate treated rats (550.53pg/ml) when compared with the control (304.42pg/ml). The high plasma concentration of IL-1β at the estrus phase of clomiphene citrate treated rats suggests its possible involvement in oocyte maturation and ovulation which characterizes the phase.

Keywords*:* Interleukin - 1β, Clomiphene citrate, Ovulation

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INTRODUCTION

The immune and physiological responses of a healthy biological system can be regulated by secretions of cytokines and chemokines from specific cell such as epithelial and endothelial cells (Saenz *et al*., 2008). These polypeptides or glycoproteins secretions have defined effects on the interactions and communications between cells. When secreted, they may act as autocrine, paracrine or endocrine. The pathway for secretion of cytokines into the microenvironment varies, granting the ability of selective management of the process of release of cytokines that will guarantee adequate and equivalent physiological response (Stanley and Lacy, 2010). Cytokine are synthesized and packaged in the Golgi complex for storage in granules or secretary vesicles and then secreted. The release of the vesicular cytokines may be by constitutive or regulated exocytosis (Jolly and Sattentau, 2007; Stow *et al*., 2009). While some cytokines (IL-2 and IL-3) possess signal sequence that mediate their trafficking via the Golgi complex, there also exist others (IL-1β and IL-18) that does not require the signal sequence for trafficking (Stanley and Lacy, 2010).

The biological effects of cytokines include immunosuppressions, cell differentiation, apoptosis and inflammation through the activities of Interferons (IFNs), Interleukins (ILs), Tumor necrosis factors (TNFs), Transforming growth factors (TGFs) (Chaouat *et al*., 2007). They have been found to influence female reproduction to a great extent by influencing fertility in mice (Rob *et al*., 1998), implantation and labour (Chaouat *et al*., 2007) and are involved in ovulation processes (Russell and Robker, 2007).

Interleukin-1 system made up of Interleukin-1α (IL-1α) and Interleukin-1β (IL-1β) are cytokines that are evolutionarily conserved with varying roles in mammalian reproduction with specific reference to uterine receptivity and implantation (Paulesu *et al*., 2010). In perfused ovaries used as model, IL-1β induced ovulation in rats (Van der Hoek *et al*., 1998); and in rabbits (Takehara *et al*., 1994). In rats, it has been found that IL-1β potentiates the inductive ovulatory effect of luteinizing hormone (LH) by

augmenting the rate of ovulated oocytes (Brannstrom *et al*., 1993).

Clomiphene citrate is a selective estrogen receptor modulator (SERM). It is widely prescribed for ovulation induction to reverse anovulation or cases of oligoovulation (Rostami-Hodjegnen, *et al*., 2004).

This study was designed to investigate the plasma concentrations of IL-1β at the different phases of the oestrus cycle of clomiphene citrate treated female wistar rats and to find a relationship between plasma concentrations of IL-1β and the phases of the oestrus cycle where possible.

MATERIALS AND METHODS

Experimental animals

A total of forty (40) female wistar rats that weighed between 150g – 255g from the animal house of the Biochemistry department of the Igbinedion University, Okada, Edo state Nigeria were used in the experiment. The animals were housed in well ventilated plastic cages with 12 hours light and dark cycle. They were fed *ad libitum* with growers' mash and water.

Experimental design

The animals were allowed to acclimatize for two weeks after grouping. Half of the female wistar rats used for the experiment was administered 0.14mg/kg clomiphene citrate while the other half which served as control received normal saline orally daily for five days. The animals were sacrificed on the sixth day between the hours of 8.00 and 10.00am after determination of the phases of oestrus cycle by microscopy. Blood samples were collected from sacrificed animals at the different phases of their oestrus cycle on the same day by cardiac puncture and put into EDTA sample bottles, centrifuged for 15 minutes at 2500rpm at 25^0C. The resultant plasma was used to determine the IL- 1β concentrations at the different phases of oestrus cycle.

Determination of Oestrus Cycle

The oestrus cycle of the female rats was determined using method of Marcondes *et al.,* (2002). While the proportion of epithelial cells, cornified cells and leukocytes were used for the determination of the oestrus cycle phases (Mandi, 1951).

Assay for Interleukin - 1β (IL-1β)

The plasma concentrations of IL-1β were determined using rat IL-1β ELISA kits (Elabscience, E-EL-R0012, 48T/24T, China).

Statistical Analysis

The results were expressed as mean ± SEM (n = number of animals). One-way Analysis of variance (ANOVA) and Chi-square were used as statistical tools. Significant differences between groups were detected in ANOVA using Bonferroni-Holm posthoc at $p<0.05$.

RESULTS

Clomiphene citrate treated and normal Female Wistar Rats at the Different Phases of the Oestrus Cycle Expressed in Percentages

The results from the virginal smear microscopy in figure 1 showed that most of the female wistar rats in both clomiphene citrate treated and control were in the estrus and diestrus phases. The percentages of clomiphene treated and control rats were found to be 10.5% and 11.8% respectively in the proestrus phase. 42.1% of clomiphene citrate treated rats were in the estrus phase compared with 29.4 % of the control which was not significant at $p<0.05$. The metestrus phase showed percentages of 10.5% and 17.7 for the clomiphene citrate and control rats respectively. Most of the control rats (41.2%) were in the diestrus phase as against 36.8% of the clomiphene citrate treated rats.

Plasma Interleukin-1β (IL-1β) Concentrations in Clomiphene Citrate Treated and Normal Female Wistar Rats at the Different Phases of the Oestrus Cycle

The results from the experiment presented in figure 2 revealed significant increases ($p<0.05$) in plasma IL-1β concentration at the proestrus and diestrus phases

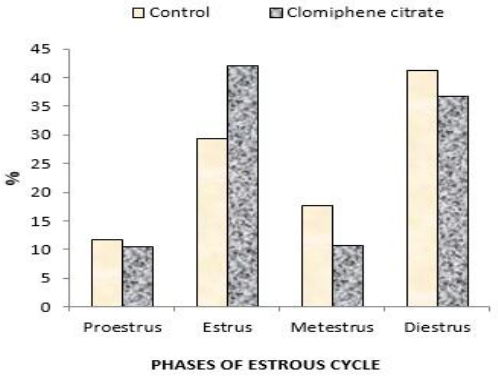

Figure 1: Percentages of clomiphene citrate treated and normal female wistar rats at the different phases of the oestrus cycle.

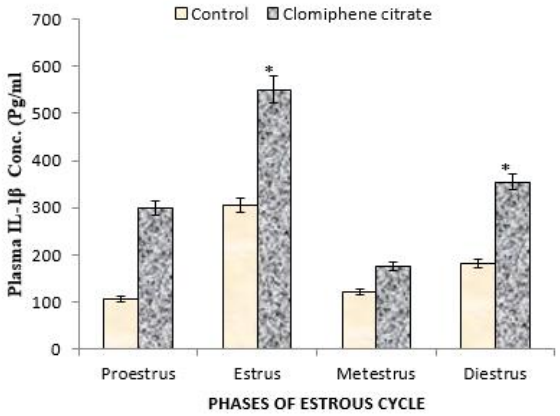

Figure 2: Plasma IL - 1β concentrations in clomiphene citrate treated and normal female wistar rats at the different phases of the oestrus cycle. *$p<0.05$

of the oestrus cycle in the clomiphene citrate treated female wistar rats when compared with the control. The increase was also significant ($p<0.05$) in the estrus phase of the clomiphene citrate treated rats (550.53pg/ml) as against the control (304.42pg/ml) in the same phase. The plasma IL-1β concentrations reached peak levels at the estrus phase for the clomiphene citrate treated female rats.

DISCUSSION

The female reproductive cycle in rats is called oestrus cycle which is characterized in phases as proestrus, estrus, metestrus and diestrus. The onset of puberty in the female rat results from pulsatile luteinizing hormone (LH) release after the fourth postnatal week (approximately thirty days of age). The change in LH release is apparent eight to nine days before the first proestrus (Westwood, 2008). From the onset of sexual maturity up to the age of 12 months, the mean cycle length in the female rat is 4 to 5 days on the average (Baligar and Kaliwal, 2001). Based on vaginal smears microscopy, the duration of the individual components of the oestrus cycle for rats with a four or five days cycle are proestrus, twelve to fourteen hours; estrus, twenty-five to twenty-seven hours; metestrus, six to eight hours; and diestrus, fifty-five to fifty-seven hours (Vom Sall *et al*., 1994; Marcondes *et al.*, 2002). It is therefore suggestive that the 41.2% of the control female rats in the diestrus phase in the experiment, though not significant ($p<0.05$) may be a function of the duration of the phase. This is in line with the work of Mandl, (1951).

The results of this work showed that 42.1% of the clomiphene citrate treated female rats were in the estrus phase of the oestrus cycle. This is unusual and can be attributed to the administration of clomiphene citrate. Clomiphene citrate is a selective estrogen receptor modulator (SERM) that blocks the negative feedback of estrogen on gonadotropin release. This lead to up-regulation of the hypothalamus – pituitary gonadal axis (Rostami-Hodjegnen, *et al*., 2004: Gadducci *et al*., 2013). The negative feedback inhibition increases the production of follicle stimulating hormone (FSH) and LH thereby increasing the tendency of ovulation that occurs at the estrus phase. LH stimulates the production of IL-1β, which in turn triggers the processes of ovulation (Fritzsche *et al*., 2006)

The peak value of plasma IL-1β as observed in this experiment may be attributed to the surge in LH that leads to increased production of reactive oxygen species that up-regulation and expression of gene. (Shkolnik *et al*., 2011). According to Russell and Robker (2007), LH acts on the ovary stimulating the synthesis IL-1β which has been shown to mimic the activities of gonadotopin at the preovulatory stage in mare (Gerard *et al*., 2004).

The high plasma concentration of IL-β at the estrus phase of clomiphene citrate treated rats in this study suggests its involvement in ovulation processes. Therefore, the effectiveness of clomiphene citrate may be its ability to stimulate the increase in the synthesis of IL-β.

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