Endocrine Disruptive Nature of Clomiphene Citrate upon the Mammalian Ovary

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1. Introduction

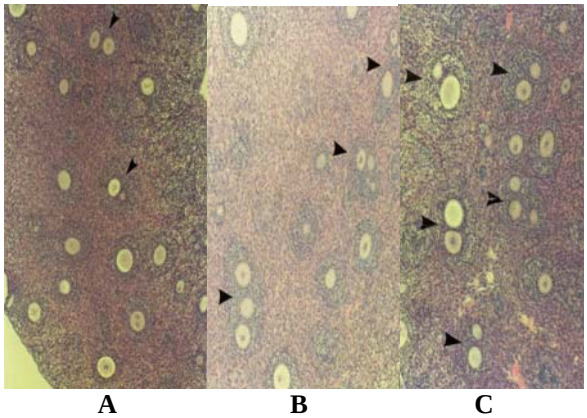
The developing mammalian female reproductive tract is particularly sensitive to actions of environmental estrogens during the period of ovarian organogenesis. Clomiphene citrate, a non-steroidal estrogen, is administered annually to thousands of women suffering from ovulatory dysfunction. Clomiphene treatment while indirectly stimulating the ovary, has a transient detrimental effect upon uterine receptivity, an effect which is eventually remediated. Clomiphene, acting via the pituitary/hypothalamic axis, stimulates the release of follicle stimulating hormone (FSH) and luteinizing hormone (LH) which induce the maturation of follicle development and ovulation. A key morphological manifestation involving fetal/neonatal exposure to endocrine disruptors is the induction of numerous polyovular follicles (POF) in the neonatal ovary, indicative of abnormal germ cell: somatic call association during primordial follicle genesis. Accordingly, interests arose to the notion whether clomiphene citrate could likewise induce POF in the ovaries of test organisms, specifically in hamsters (1-3).

2. Materials and Methods

Expecting female hamsters were received prior to their delivery date. Within 6 hours of birth, female and male hamsters were separated. Upon separation, female neonatal hamsters received a single injection of warm saline (50 ul, sc) or saline containing 1, 10, or 100 ug clomiphene citrate. Five pups were injected per group then returned to their mother. At 21 days of age, pups were anesthetized by CO^2 and decapitated. One of the paired ovaries were removed and placed into Z Fix fixative and stored overnight. The second ovary proceeded to be snap frozen for eventual RNA preparation. The following day ovaries were treated with fresh Z Fix and then eventually removed and replaced by 70% ethanol. Ovaries that were fixed were sent to Histo-Scientific Laboratories (Woodstock, VA) for embedding and sectioning. The five control ovaries were aligned in the tissue block such that they could be sectioned simultaneously. Ovaries were serially sectioned and after the first 10 sections, every 12^{th} subsequent section was placed on a slide and stained. The same procedure was repeated with the clomiphene ovaries. Sixteen sections were obtained for each group, (i.e. 16/212 of the total sections were analyzed or 7.5% of the total ovary). For both the control and clomiphene groups, each section was analyzed for the total number of developing follicles and the number of polyovular follicles. Data were analyzed by one way ANOVA with multiple comparisons, and expressed as total number of developing follicles per ovary, POF per ovary, and POF/number of developing follicles.

3. Results

For both control and clomiphene groups, each section of each of the 5 ovaries was analyzed for total number of developing follicles and the number of POFs (figure 1). Sixteen sections were obtained for each group, thus 16/212 of the total sections were analyzed, equaling 7.5% of the total ovary. For all groups, each section was analyzed for total number of developing follicles and POF. The total number of developing follicles counted in the 16 sections among the control and 1, 10, and 100 ug CC groups was not statistically different (463 +/- 34, 514 +/- 20, 470 +/- 25, and 418 +/- 23, respectively)(figure 2). However, when the number of POF was determined (25 +/- 2, 28 +/- 5, 44 +/- 3, and 46 +/- 5), the 10 and 100 ug CC doses exhibited significantly elevated numbers of POF ($P<0.05$) (figure 3). In relating POF to total follicles per ovary (0.06 +/- 0.002, 0.05 +/- 0.008, 0.09 +/- 0.007, and 0.11 +/- 0.011), the two higher CC doses exhibited a significant and dose-dependent increase ($P< 0.05$ and 0.01, respectively) (figure 4). Microscopically speaking, the origins of the POFs seen within the various clomiphene treated ovaries reveal to be contained within the inner portions of the ovary
(figure 5). The development state of these POFs are relatively unknown.



A **B** **C**

Figure 1 Dose effect of clomiphene citrate (CC) upon polyovular follicle (POF) formation. (A) Control, most follicles are round and contain a single oocyte. However, POF are present (arrowheads). (B) and (C); POF in ovaries from animals treated with 10 and 100 ug CC.

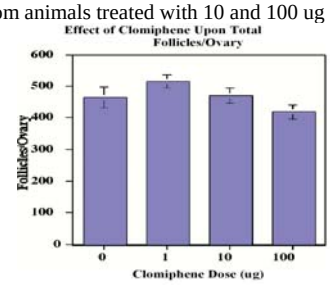


Figure 2 Dose effect of CC upon the number of developing follicles in the ovary. Developing follicles are defined as primary or greater.

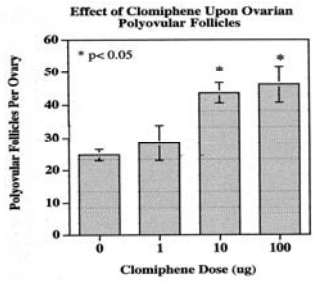


Figure 3 Dose effect of CC upon the number of ovarian POF.

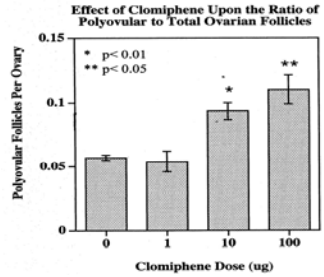


Figure 4 Dose effect of CC upon the number of POF relative to the number of total developing follicles.

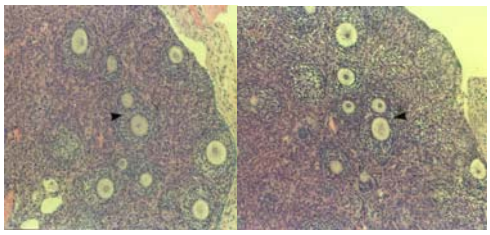


Figure 5 Origins of POF. Note the follicles indicated (arrowheads), which suggest that POF are forming by fusion, and located in the central region of the ovary

4. Discussion

POF development produces abnormal, oblong follicles that generally contain two or more oocytes, as apposed to single oocyte seen within normal follicles. In a normal ovary (untreated) the appearance of POFs are minimal to rare. However, relative to controls, CC given at 10 and 100 ug doses significantly increases the number of POF per ovary. In relating these POFs to their originality, the number of total follicles within the various doses remained unchanged in number. The orientation of these POFs suggests that POF formation could arise early in development by failure of oocytes to separate as they attract mesothelial cells from the genital ridge. Conversely, POFs could arise from fusion of developing follicles. The data suggest, at least preliminarily, that clomiphene citrate has endocrine disruptive characteristics similar to other more recognized disruptors such as diethylstilbestrol (DES).

5. Conclusion

In observing the results upon clomiphene dose response, apparent detection of an increased number of POF developing within the ovary becomes amplified in conjunction to neonatal exposure of this compound. Interestingly, in response to clomiphene, POFs became distinctly congregated to the inner, central portion of the ovary. Additional investigation onsite of these POFs show the apparent development of this abnormal state by the fusion of individual, otherwise normal follicles with one another.

6. Acknowledgements

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7. References

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