

EVALUATION OF CLOMIPHENE CITRATE-INDUCED ANTI-ESTROGENIC EFFECTS ON DEVELOPMENT AND MATURATION OF GUINEA PIG OOCYTES

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General Objective:

Evaluation of Clomiphene Citrate (CC) induced anti-estrogenic effects on development and maturation of guinea pig oocytes.

Specific Objectives:

1. Morphological evaluation on development and maturation of oocytes in vivo and in vitro.
2. Evaluation of biochemical parameters such as estradiol, cAMP and PGE2 levels in blood and ovary.
3. Evaluation of egg survival/ death parameters such as Bax/Bcl2 expression, caspase-3 activation and DNA fragmentation.
4. Evaluation of fertilization and developmental ability of eggs in control and Clomiphene Citrate treated groups.
5. Study of dose and time for E2 supplementation and to see its protective effects on the above specific objectives.

Methodology:

a) Study population:

Sexually mature female guinea pigs having regular estrous cycle were used for the experimentation.

Protocol for study: Sexually mature female guinea pigs with regular cycle have been subjected to various treatment protocols. The vehicle, estradiol, CC with or without estradiol were administered for 3 days intramuscularly during pro-estrous phase of the cycle. For in vivo studies, clomiphene citrate was initially dissolved in absolute alcohol and then diluted in olive oil (2.5, 5.0, 10.0, and 20.0 mg/mL) for the preparation of intra-peritoneal injections. All animals of 4 main groups namely vehicle control, E2 (1 mg / kg bd wt) alone, CC (0, 2.5, 5.0, and 20 mg/kg bw) alone and CC+ E2 was given. Respective interventions thereafter are carried out. Ovary along with oviduct were taken out from animals and processed for-

1. Histology and immunohistochemistry of ovary.
2. Estimation of E2 and NO in circulation, and PGE2, and cAMP levels in the ovary.

3. Analysis egg survival by morphological and biochemical changes.
4. Analysis of developmental ability of fertilized ovum in vitro.

Progress:

1. Evaluation of biochemical parameters such as estradiol, cAMP and PGE2 levels in blood and ovary was carried out.
2. Study of dose and time for E2 supplementation and to see its protective effects on the above specific objectives.
3. Analysis of fertilization ability in vivo was studied.
4. The experimental work has been completed.
5. Results of the data analysed -
 - a) No significant change in the body weight and organ weight.
 - b) The effective dose of C.C. is 10 mg/kg bd wt.
 - c) The blood estrogen levels were reduced by CC alone.
 - d) Ovarian cAMP activity is increased with CC.
 - e) PGE2 levels were reduced in all treatment groups.
 - f) NO activity is reaches maximum with CC plus Estrogen.

Summary of Results so far:

The data analysis is under process and the complete report will be submitted in the next PAC meeting.

Budget:

	1 st Year	2 nd Year	3 rd Year	Total
Equipment (Osmometer)	1,00,000			1,00,000
Chemicals	1,00,000	1,00,000	1,00,000	3,00,000
Project Assistant/ Technical Assistant Rs.7000/- consolidated for 30 months.	84,000	84,000	42,000	2,10,000
Total				6,10,000

Any other Comments: Nil