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ScienceDirect
Life Sciences 79 (2006) 2217–2220
Life Sciences
www.elsevier.com/locate/lifescie

Ovulation induction by antiestrogens in an Indian tropical vespertillionid bat, *Scotophilus heathi*

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Received 18 April 2006; accepted 17 July 2006

Abstract

The ovulation induction property of ICI 182,780 a pure antiestrogen and enclomiphene citrate (ENC) was carried out in *Scotophilus heathi*, an Indian tropical vespertillionid bat, during December to February i.e., preovulatory period. This bat ovulates two ova naturally and shows ovulatory asynchrony. The study showed that 100 ìg of ENC followed by 10 IU hCG resulted in significantly lower number of ovulation. Whereas, the pure antiestrogen ICI 182,780 at a dose of 100 ìg followed by 10 IU hCG resulted in ovulation induction (4.2±0.4), which is significantly different in comparison to other groups. This is possibly the first report of ovulation induction using this pure antiestrogen i.e., ICI 182,780 in any bat as well as in any animal model that exhibits temporary anovulation similar to polycystic ovary disease (PCOD). This antiestrogen may be useful to induce ovulation in PCOD patients.

Keywords: Ovulation induction; Antiestrogen; ICI 182, 780; Enclomiphene citrate

Introduction

In *Scotophilus heathi*, the ovaries during the preovulatory period (Dec.–Feb.) show a close resemblance with that of PCOD females (Abhilasha and Krishna, 1996) with prolonged survival of Graafian follicle. PCOD is characterized by chronic anovulation with an excess of estrogen, mainly estrone, due to extragonadal aromatization of circulating androstenedione. It has been established that patients with PCOD show a positive response to clomiphene citrate (March and Mishell, 1986; Speroff et al., 1979; Franks, 1989; McKenna, 1988). From our laboratory it has been previously shown that clomiphene citrate can induce ovulation in *S. heathi* during the period of preovulatory period (Kumar and Pakrasi, 2000).

Previously, we reported that clomiphene citrate (CC) and its two isomers induced ovulation to different degrees. We found that CC along with 10 IU hCG was most effective where as CC alone failed to induce ovulation. In mice we reported that the CC isomer enclomiphene citrate (ENC) was more potent than zuclomiphene (ZNC) isomer in ovulation induction (Pakrasi and Kumar, 2001). In the present report we have used only ENC isomer and a new pure antiestrogen to check the effect on bat model. In this study enclomiphene citrate (ENC) and ICI 182,780 was used to induce ovulation in *S. heathi*. ENC is *trans*-isomer of clomiphene citrate and accounts for its ovulation induction activity in the racemic mixture. ICI 182,780 is a potent specific

Table 1
Induction of ovulation by ICI 182,780 and enclomiphene citrate (ENC) during the preovulatory period in *S. heathi*

Group[a]	Treatment	Number of ovulations[b]	Ovarian weight[c] mg
1.	Control saline	0	2.11±0.06
2.	10 IU hCG	0.6±0.5	2.41±0.01
3.	10 IU ENC	0	2.14±0.01
4.	10 IU ICI 182,780	0	2.11±0.01
5.	100 μg ENC+10 IU hCG	1.4±0.5	3.78±0.02
6.	100 μg ICI 182,780+ 10 IU hCG	4.2±0.4	4.62±0.30

[a] 5 animals in each group.
[b] Values are mean±SEM. All the groups are significantly different at the level $P<0.01$, except Group 1 vs. Groups 3 and 4.
[c] Values are mean±SEM. All the groups are significantly different at the level $P<0.01$, except Group 1 vs. Groups 3 and 4.

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0024-3205/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.lfs.2006.07.026

pure antiestrogen, which is widely used in breast cancer treatment compared with partial agonists like tamoxifen (Dukes et al., 1992). Pharmacologically ICI 182,780 blocks the uterotropic action of estradiol in a dose dependent manner. This action shows similarity with the action in rodents (Dukes et al., 1992). ICI 182,780 completely neutralized the uterotropic response of E_2 in rodents when injected along with estrogen (Parisot et al., 1995).

Previous studies on the induction of ovulation in bats have been achieved with gonadotropic hormones of pituitary or chorionic origin (Wimsatt, 1960; Racey, 1976; Racey, 1982).

Material and methods

Animals

Female mature bats weighing 35–40 g were captured live from adjacent areas of Varanasi during the preovulatory period (i.e., December to February). They were housed in a temperature and light controlled (14 L:10 D) animal room. They were maintained in cages with free access to water and were provided with live insects.

Experimental design

Female bats were assigned to several groups irrespective of their size. Five animals were used in each group. Antiestrogen ICI 182,780 was dissolved in alcohol whereas and ENC was dissolved in DMSO and diluted in 0.9% saline. All the injections were given intraperitoneally to each bat. Group 1 and 2 were controls. Animals of Group 1 received only saline and group 2 served as the hCG (Chorulon, Intervet International B. V., Boxmeer, Holland) control. Group 3 and 4 received ENC and ICI 182,780 at 100 μg doses alone, respectively. Group 5 received ENC at 100 μg followed by 10 IU hCG after 48 h. Group 6 was supplied with 100 μg ICI 182,780 followed by 10 IU hCG after 48 h. Animals were sacrificed 18 h after hCG treatment with excess of ether. The

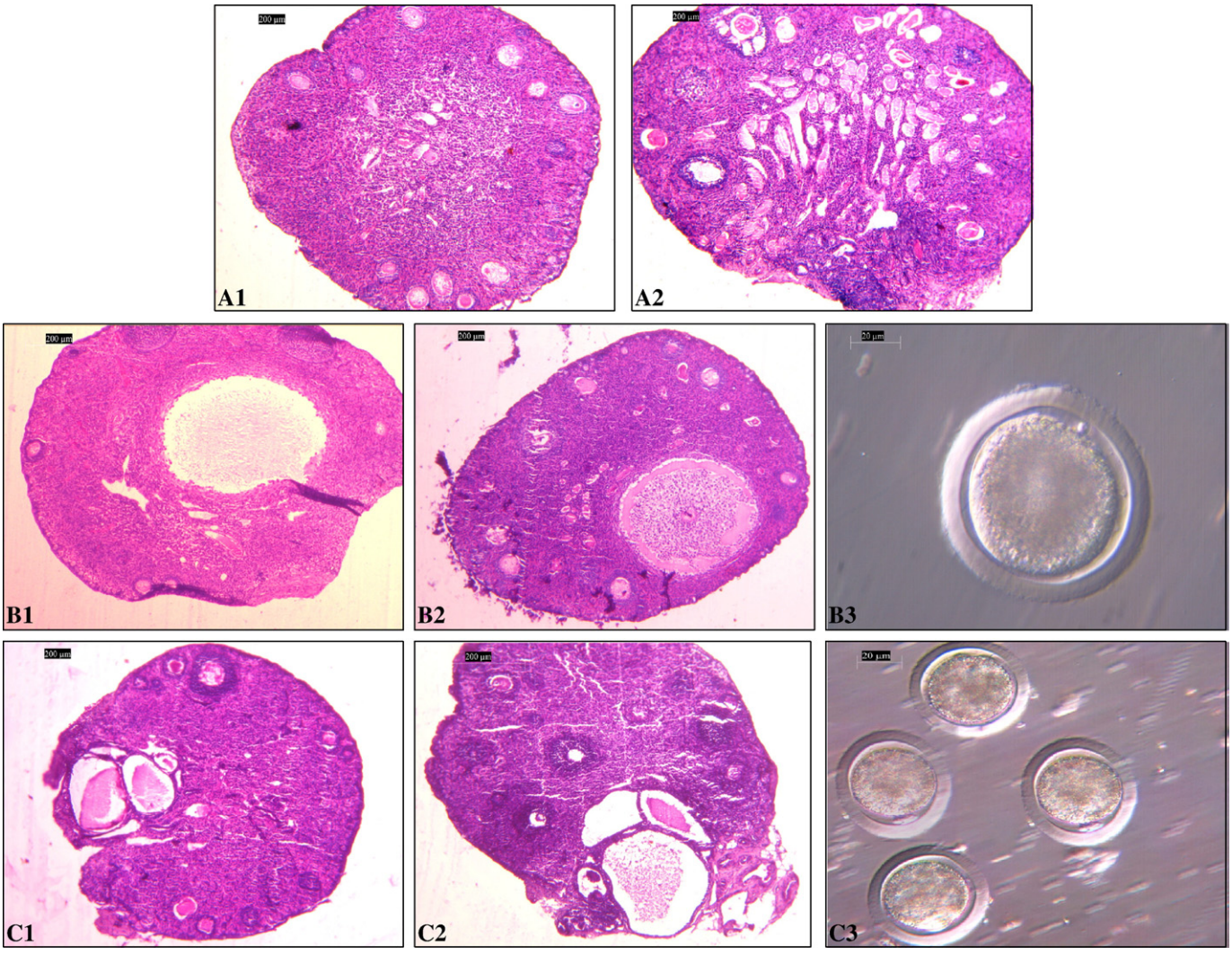

Fig. 1. Ovulation induction in *S. heathi* using enclomiphene citrate and ICI 182, 780. Upper panel is vehicle treated control right (A1) and left (A2) ovaries. Middle panel is enclomiphene citrate+10 IU hCG treated right (B1) and left (B2) ovaries. Lower panel is ICI 182,780+10 IU hCG treated right (C1) and left (C2) ovaries. Control ovaries shows unstimulated scattered cortical follicles. The histology of the middle panel shows only one ovulation (B1). However, follicle maturation was stimulated (B2) showing super mature Graafian follicle. B3 is the resulted ovum ovulated from B1. C1 and C2 is the first ever microphotographs showing ovulation induction from any bat ovary. C3 is the ovulated ova from one bat treated with ICI 182,780.