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Theriogenology
ELSEVIER
Theriogenology 61 (2004) 393–398

Effects of a GnRH agonist on oocyte number and maturation in mice superovulated with eCG and hCG

Mehmet Kanter[a], Cengiz Yildiz[b], Ismail Meral[c,*], Ahmet Koc[a], Ibrahim Tasal[b]

[a]*Department of Histology and Embryology, Faculty of Veterinary Medicine, Yuzuncu Yil University, 65080, Kampus, Van, Turkey*
[b]*Department of Reproduction, Faculty of Veterinary Medicine, Yuzuncu Yil University, 65080, Kampus, Van, Turkey*
[c]*Department of Physiology, Faculty of Veterinary Medicine, Yuzuncu Yil University, 65080, Kampus, Van, Turkey*

Received 5 March 2002; accepted 15 May 2003

Abstract

The objective was to investigate the effects of a gonadotropin-releasing hormone agonist (GnRH) on ovulation rate and the number and maturation of oocytes in mice superovulated with equine chorionic gonadotropin (eCG) and human chorionic gonadotropin (hCG). Thirty 3-month-old BALB/C female mice (weight: 25–30 g) were assigned to three experimental groups: control, superovulated, and superovulated with GnRH pretreatment ($n = 10$ per group). Control mice received an i.p. injection of 0.1 ml physiological saline solution. Superovulation was induced with 5 IU eCG (i.p.) and 5 IU hCG 48 h later. Mice in the superovulated with GnRH pretreatment group were given GnRH (20 mg/kg Fertirelin, i.m.), 24 h before superovulation. Thirteen hours after hCG administration, mice were sacrificed by cervical dislocation and blood samples were collected to determine serum progesterone concentration (by radioimmunoassay). Ovaries and oviducts were also harvested to enumerate corpora lutea and cumulus-enclosed oocytes. Progesterone concentrations were not significantly different among groups. The oocyte number and the maturation, ovulation rate, and the number of corpora lutea were higher in GnRH-treated mice than both controls and superovulated mice. In conclusion, GnRH given 24 h before superovulation with eCG–hCG increased the number and maturation of oocytes and the rate of ovulation in mice.

Keywords: GnRH agonist; Superovulation; Oocyte maturation; Mice

* Corresponding author. Tel.: +90-432-2251128; fax: +90-432-2251127.
E-mail address: imeral20@hotmail.com (I. Meral).

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doi:10.1016/S0093-691X(03)00222-X

1. Introduction

Gonadotropin-releasing hormone agonists (GnRHs) are used clinically as adjunctive therapy during ovarian stimulation [1]. The effects of GnRH on the rat ovary include stimulation of oocyte maturation [2,3]. However, a direct gonadal effect of GnRH may interfere with the stimulatory effects of exogenous gonadotropin on follicular development, corpus luteum establishment, and oocyte maturation [1].

The follicular oocyte in mammals is arrested at the diplotene stage of prophase of the first meiotic division. The physiological stimulus for the resumption of meiosis is the preovulatory surge of luteinizing hormone (LH) [4,5]. Native GnRH and its analogs may mimic LH action, thereby inducing resumption of meiosis; in that regard, in vitro exposure of isolated ovarian follicles to GnRH (or its agonists) resulted in oocyte maturation [6,7].

The primary objective of this study was to investigate the effects of a GnRH on ovulation rate, and the number and maturation of oocytes in mice superovulated with eCG and hCG. Furthermore, since it has been suggested that GnRH stimulates development of the preovulatory follicle-enclosed oocytes, leading to progesterone synthesis in granulosa cells [8], serum progesterone concentrations were also determined.

2. Materials and methods

Thirty 3-month-old BALB/C female mice (weight, 25–30 g) were used. Feed and water were available ad libitum. In order to maintain stable biological rhythms, 12 h of artificial light and 12 h of darkness were provided. All mice received humane care according to the criteria outlined in the ''Guide for the Care and Use of Laboratory Animals'' prepared by the National Academy of Sciences and published by the National Institutes of Health.

The mice were assigned to one of three experimental groups: control, superovulated, and superovulated with GnRH pretreatment ($n = 10$ per group). Control mice received only 0.1 ml 0.9% saline (i.p.) at the same time as the eCG and hCG injections were given to the two superovulated groups. Superovulated mice were given an i.p. injection of 5 IU of equine chorionic gonadotropin (eCG; Folligon®, Intervet, Istanbul, Turkey), followed 48 h later by 5 IU of human chorionic gonadotropin (hCG; Pregnyl®, Organon, Istanbul, Turkey). In addition, the GnRH-treated mice received 20 mg/kg (i.m.) of a GnRH agonist (Fertirelin acetate: Pro-His-Trp-Ser-Tyr-D-Ser-Leu-Arg-Pro-NH-CH_2-CH_3, Ovalyse®, Eczacibasi, Istanbul, Turkey) 24 h before superovulation with eCG–hCG.

Thirteen hours after hCG administration, the mice were sacrificed by cervical dislocation and blood samples were taken for determination of serum progesterone concentrations by radioimmunoassay (IMMULITE® 2000, Diagnostic Products Corporation, Los Angeles, CA). The ovaries and oviducts were harvested and put into a human tubal fluid (HTF)-medium [9]. The number of corpora lutea on the ovaries was counted using a stereo microscope (Nikon SMZ800, Japan). The oviducts were excised and the cumulus-enclosed oocytes were recovered from the tubes into HTF-medium containing 0.5 mg/ml hyaluronidase (Sigma Co., St. Louis, MO). Oocytes were removed from the cumulus cells by gentle agitation using a narrow pipette and were placed in fresh HTF-medium; the number and maturity of the oocytes were determined with an inverted microscope

(Nikon T300, Japan). Oocyte maturity was based on the observation of a polar body. Mature oocytes were referred to as metaphase II oocytes. An immature oocyte (metaphase I) was defined by the presence of a germinal vesicle.

The ovulation rate was calculated as the number of oocytes recovered from the oviducts as a percentage of the number of corpora lutea in the ovaries. Data were analyzed with analysis of variance (ANOVA). If there was an effect ($P < 0.05$) of treatment group, Duncan's test was used to locate differences.

3. Results

The number of mature oocytes and the ovulation rate were higher ($P < 0.05$) in the GnRH-treated mice than the superovulated group and controls (Table 1). In the GnRH-treated group, 86% of oocytes were in metaphase II (mature oocyte with polar body) and 14% of oocytes were in metaphase I (immature oocyte with germinal vesicle). In the superovulated and control groups, only 58% of oocytes were mature (oocyte II) and 42% of oocytes were immature (oocyte I). Oocytes obtained from all groups were morphologically normal. The numbers of oocytes and corpora lutea were higher ($P < 0.05$) in the GnRH-treated group compared to those of the controls and superovulated groups (Figs. 1 and 2,

Table 1
Numbers of mature and immature oocytes, and ovulation rate in the control and treatment groups ($n = 10$ per group)

Parameters of oocytes	Control (NaCl)	GnRH-treated (GnRH + eCG–hCG)	Superovulated (eCG–hCG)
Immature (mean ± S.D., range)	7.6 ± 1.2^{a} (4–9)	4.1 ± 0.5^{b} (3–5)	8.7 ± 0.9^{a} (7–11)
Mature (mean ± S.D., range)	6.2 ± 1.0^{a} (4–8)	25.2 ± 3.2^{c} (22–29)	12.0 ± 1.2^{b} (9–14)
Ovulation rate (%)	82.3 ± 2.2^{a}	94.8 ± 3.1^{b}	89.1 ± 2.4^{c}

Within a row, groups with different superscripts (a and b) are different ($P < 0.05$).

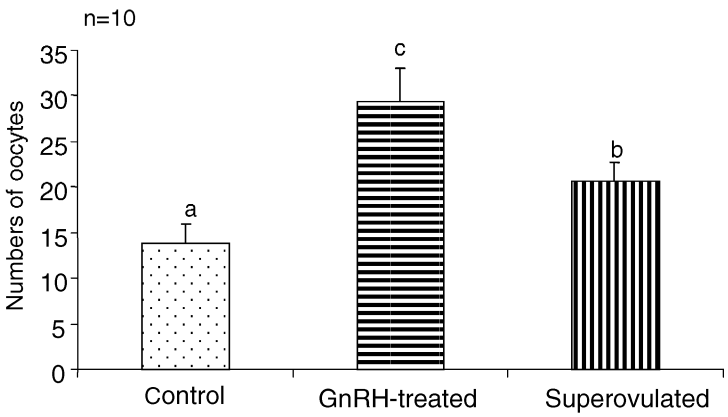

Fig. 1. Numbers of oocytes recovered from the oviducts of the control and treatment groups. Different letters indicate a difference ($P < 0.05$) between groups. Data are expressed as mean ± S.D.

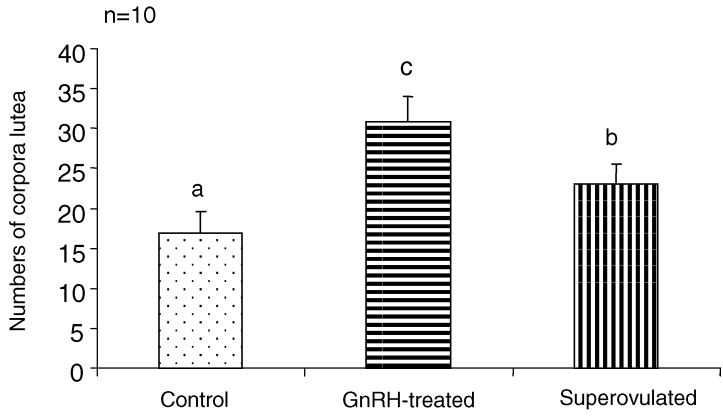

Fig. 2. Number of corpora lutea (combined for both ovaries) in the control and treatment groups. Different letters indicate a difference ($P < 0.05$) between groups. Data are expressed as mean ± S.D.

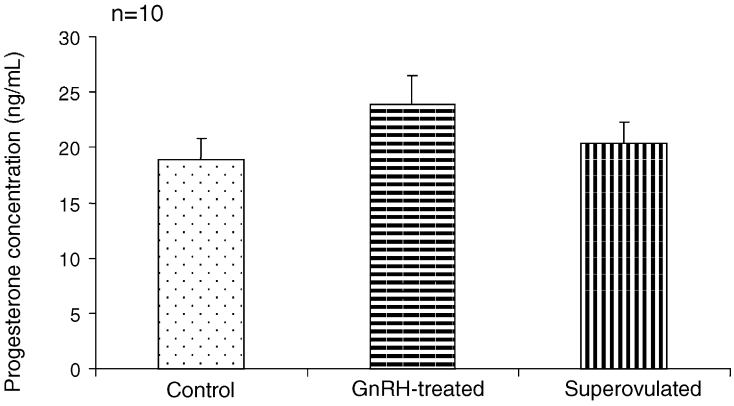

Fig. 3. Serum progesterone concentrations in the control and treatment groups. Different letters indicate a difference ($P < 0.05$) between groups. Data are expressed as mean ± S.D.

respectively). Serum progesterone concentrations were not different ($P > 0.05$) among groups (Fig. 3).

4. Discussion

Pretreatment with GnRH prior to superovulation significantly increased the number of oocytes recovered, compared to superovulation without GnRH. It has been shown that GnRH mediates the hypothalamic control of pituitary gonadotropin secretion and biosynthesis [2]. However, recent studies have shown that, at least in mice, rats and rabbits, GnRH and its analogues also exert a direct effect on gonadal function, affecting or

influencing oocyte maturation both in vivo and in vitro [3,10]. It has been suggested that GnRH induces oocyte maturation via activation of specific GnRH receptors on granulosa cells [2,11]. It has also been suggested that exposure to GnRH stimulates prostaglandin (PG) E_2 and $PGF_{2\alpha}$ synthesis in preovulatory follicles [12]. Increasing concentration of PGs play an important role in oocyte maturation [13]. Alternatively, GnRH stimulates re-initiation of meiosis by direct interaction with the oocyte [14,15]. There is a correlation between increased PG concentrations and the resumption of meiosis and oocyte maturation; the association between the resumption of meiosis and the loss of cumulus–oocyte contact has prompted speculation that PGs may alter the cumulus–oocyte association, resulting in the induction of meiotic maturation [16].

Since GnRH does not influence cyclic AMP accumulation in rat granulosa cells or in rabbit preovulatory follicles, it appears to stimulate PG accumulation by a mechanism that does not involve cyclic AMP [12]. Thus, direct gonadal effects of GnRH may represent secondary pharmacological properties. The increased number of mature oocytes in the GnRH-treated group in the present study also supported the idea that GnRH stimulated the resumption of meiosis in ovarian follicles. However, since we did not measure cyclic AMP concentration, we do not know whether it played a role in the GnRH-induced increase in oocyte number and maturation.

There was no significant effect of treatment group on serum progesterone concentrations. Yang et al. [3] studied the effects of a GnRH on oocyte maturation, fertilization, and embryonic development in mice. Similar to our results, they found that although the number of oocytes obtained from the GnRH-treated mice was higher, progesterone concentration was not different from controls. It has been suggested that exposure to a GnRH, at a dose that induces the resumption of meiosis, does not stimulate progesterone increase [7].

In conclusion, an intramuscular injection of GnRH 24 h before superovulation with eCG–hCG increased oocyte number and maturation in mice. The increased number and maturity of oocytes found in the GnRH-treated mice may have implications for in vivo and in vitro fertilization techniques; increased numbers of mature oocytes will increase the chance of fertility. Thus, GnRH treatment 24 h before superovulation with eCG–hCG should be further studied to assess potential applications for in vivo and in vitro fertilization.

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