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Original Research Article

Effect of recombinant-LH and hCG in the absence of FSH on *in vitro* maturation (IVM) fertilization and early embryonic development of mouse germinal vesicle (GV)-stage oocytes

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ARTICLE INFO

Article history:
Received 15 September 2015
Received in revised form
18 January 2016
Accepted 22 January 2016
Available online 4 February 2016

Keywords:
IVM
r-LH
hCG
GV
LH/hCG receptor

ABSTRACT

During *in vitro* maturation (IVM), intrinsic and extrinsic factors must co-operate properly in order to ensure cytoplasmic and nuclear maturation. We examined the possible effect of LH/hCG in the process of oocyte maturation in mice with the addition of recombinant LH (r-LH) and hCG in our IVM cultures of mouse germinal vesicle (GV)-stage oocytes. Moreover, the effects of these hormones on fertilization, early embryonic development and the expression of LH/hCG receptor were examined. Nuclear maturation of GV-stage oocytes was evaluated after culture in the presence of r-LH or hCG. Fertilization rates and embryonic development were assessed after 24 h. Total RNA was isolated from oocytes of different stages of maturation and from zygotes and embryos of different stages of development in order to examine the expression of LH/hCG receptor, using RT-PCR. The *in vitro* nuclear maturation rate of GV-stage oocytes that received hCG was significantly higher compared to the control group. Early embryonic development was increased in the hCG and LH cultures of GV oocytes when LH was further added. The LH/hCG receptor was expressed in all stages of *in vitro* matured mouse oocytes and in every stage of early embryonic development. Addition of hCG in IVM cultures of mouse GV oocytes increased maturation rates significantly. LH, however, was more beneficial to early embryonic development than hCG. This suggests a promising new technique in basic science research or in clinical reproductive medicine.

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http://dx.doi.org/10.1016/j.repbio.2016.01.004

1. Introduction

In vitro maturation (IVM) of mouse oocytes provides not only the means to produce a uniform population of developmentally competent oocytes, but also holds promise for applications in basic laboratory settings. More than two decades have passed since the report of the first live mouse offspring using IVM. The same technique has been widely used in several mammalian species and was applied clinically in human assisted reproductive technology, leading to live-born offspring using human oocytes after IVM [1,2]. *In vivo*, human oocytes are arrested at prophase in meiosis I (MI) for 12–50 years before ovulation. This pre-ovulation stage involves an intricate process of dominant follicle selection followed by nuclear maturation, germinal vesicle (GV) breakdown, chromosomal arrangement, and completion of MI by extrusion of the first polar body, all of which occur concurrently with a not so well characterized process of cytoplasmic maturation. Likewise, during IVM, intrinsic and extrinsic environmental factors such as hormones, growth factors, and nutrients must co-operate properly to ensure cytoplasmic and nuclear maturation.

There are many clinical benefits regarding IVM despite certain pitfalls. In *in vitro* fertilization (IVF) the number of collected oocytes increases by using external gonadotropins for ovarian stimulation. Unfortunately, stimulation protocols increase the chance of ovarian hyperstimulation syndrome (OHSS) [3]. The amount of external gonadotropin administration is reduced during IVM, thus minimizing the risk of hyperstimulation. IVM is also proposed as a solution to preserve fertility [4] concerning patients with polycystic ovary syndrome [5,6] and patients with a high risk of future infertility, such as young females undergoing aggressive gonadotoxic chemotherapy [7]. Recent reports have described the use of IVM for oocytes retrieved from antral follicles with subsequent successful fertilization and pregnancy [8,9]. Despite previous data demonstrating low pregnancy outcome and caution in IVM indications, innovative findings in this field have opened new horizons in the treatment of patients [10]. In addition, GV oocytes obtained during an IVF/intracytoplasmic sperm injection (ICSI) cycle are not used clinically and are usually discarded. Although some of these oocytes may be atretic or may have been resistant to the *in vivo* gonadotropin stimulus, some are still capable of undergoing maturation and fertilization, if appropriate conditions are present *in vitro* [11]. Hence, mouse oocytes could be used to study and optimize the culture conditions for human IVM.

Luteinizing hormone (LH) and human chorionic gonadotropin (hCG) are integral components of the hypothalamic–pituitary–gonadal axis, which controls sexual maturation and functionality. LH is a key regulator of gonadal steroidogenesis and ovulation, in contrast to hCG that is predominantly active in pregnancy and fetal development. However hCG is considered the wonder of today's science [12], since it is the most acidic glycoprotein containing the highest proportion of sugars and is involved in functions ranging from control of human pregnancy to human cancer. During assisted reproductive technology (ART) protocols, hCG has been used as a surrogate for the natural midcycle LH surge. Due to the structural and biological similarities, both hCG and LH activate and bind to the same receptor, the LH/hCG receptor (LHCGR) [13]. A critical difference, however, occurs regarding the half-life of LH and hCG; the half-life of LH is approximately 60 min [14], while that of hCG exceeds 24 h [15].

Granulosa cells of primary follicles express FSH receptors (FSHR), while the theca cells of secondary follicles express LH/hCG receptors [16,17]. During ovulation, most probably under the influence of LH/hCG, the main target cells for LH/hCG switch from theca interna cells/small luteal cells to granulosa cells/large luteal cells [18]. The binding of gonadotropins to their G-protein-receptor activates adenyl cyclase and induces an intracellular rise in cyclic AMP levels [19]. Our studies showed that FSH and LH receptors' messenger RNA was also observed in both human and mouse oocytes (metaphase II), indicating a physiological role in the oocyte maturation [20,21].

Comparing the use of recombinant LH and hCG in oocyte maturation during clinical IVM permits us to investigate the differences between the effects of these gonadotropins in a well-designed *in vitro* system. In oocytes matured *in vitro*, recombinant FSH and hCG are added to the culture medium, and maternal serum is also used as a supplement in clinical IVM programs [22]. However, the cellular response upon FSH cannot entirely substitute for LHCGR signaling during the final stages of follicle growth and ovulation as shown in the LHCGR knockout mouse [23] and in women with inactivating mutations of LHCGR [24].

Many studies have creatively found ways to use animal model experiments, *in vitro* or *in vivo*, to analyze the effects of gonadotropins on mammalian oocyte maturation and this kind of research is expected to provide further support for their clinical application [25,26]. Given the importance of the use of both LH and hCG in *in vitro* maturation systems during ART, the aim of the current study was to assess the effect of LH and hCG addition to the IVM culture medium of mouse GV-stage oocytes before and after fertilization through early embryonic development. Furthermore, we examined the expression of LH/hCG receptor mRNA at both mouse oocytes and early embryos using RT-PCR.

2. Materials and methods

2.1. Experimental animals

All female and male mice used in this study were (C57BL/6♀ × CBA♂) F1 hybrids raised and cared at the Pasteur Institute (Athens, Greece). This study was reviewed and approved by the University Hospital Ethics Committee and the Animal Care and Use Committee of the Pasteur Institute (Ethics Committee of the ''Alexandra Hospital'', Reference number: 662, Date: 4/12/14).

2.2. Experimental groups

2.2.1. *In vitro maturation of mouse oocytes*

In order to assess the effect of LH and hCG on *in vitro* maturation of mouse GV-stage oocytes, three experimental groups were studied:

(A) *Control group*: GV-stage oocytes cultured *in vitro* in the basic culture medium.