

Meiotic Arrest in Human Oocytes Is Maintained by a G_s Signaling Pathway¹

Andrea DiLuigi,^{3,4} Vanessa N. Weitzman,^{3,4} Margaret C. Pace,³ Linda J. Siano,⁴ Donald Maier,⁴
and Lisa M. Mehlmann^{2,3}

Department of Cell Biology³ and Center for Advanced Reproductive Services,⁴ University of Connecticut Health Center,
Farmington, Connecticut 06030

ABSTRACT

In mammalian oocytes, the maintenance of meiotic prophase I arrest prior to the surge of LH that stimulates meiotic maturation depends on a high level of cAMP within the oocyte. In mouse and rat, the cAMP is generated in the oocyte, and this requires the activity of a constitutively active, G_s -linked receptor, GPR3 or GPR12, respectively. To examine if human oocyte meiotic arrest depends on a similar pathway, we used RT-PCR and Western blotting to look at whether human oocytes express the same components for maintaining arrest as rodent oocytes. RNA encoding GPR3, but not GPR12, was expressed. RNA encoding adenylylase type 3, which is the major adenylylase required for maintaining meiotic arrest in the mouse oocyte, was also expressed, as was $G\alpha_s$ protein. To determine if this pathway is functional in the human oocyte, we examined the effect of injecting a function-blocking antibody against $G\alpha_s$ on meiotic resumption. This antibody stimulated meiotic resumption of human oocytes that were maintained at the prophase I stage using a phosphodiesterase inhibitor. These results demonstrate that human oocytes maintain meiotic arrest prior to the LH surge using a signaling pathway similar to that of rodent oocytes.

cyclic adenosine monophosphate, gamete biology, signal transduction

INTRODUCTION

Mammalian oocytes are stored in the ovary arrested at prophase I of meiosis. Throughout the reproductive period of the female, ovarian follicles grow in response to stimulation by the pituitary gonadotropin FSH. Oocyte growth occurs concomitantly with follicle growth, but the oocyte remains arrested at prophase I until a preovulatory surge of LH from the pituitary stimulates meiotic resumption. The prophase I-arrested oocyte acquires the ability to resume meiosis as it approaches its full size. In response to LH, the oocyte resumes meiosis and progresses to metaphase II, at which point it becomes arrested again and is at the appropriate stage to be fertilized. The progression from prophase I to metaphase II is termed oocyte maturation and is a process that includes nuclear as well as cytoplasmic changes that allow the mature egg to be fertilized. The LH surge that initiates meiotic resumption also stimulates ovulation, and these two events are coordinated such

that by the time the oocyte is ovulated, it has completed the maturation processes necessary to produce a fertilizable egg.

Meiotic arrest in fully grown, meiotically competent oocytes is dependent on high levels of cAMP within the oocyte [1, 2]. In rodent oocytes, cAMP is generated in the oocyte through the activity of a G-protein coupled receptor, GPR3 (mouse) or GPR12 (rat), that activates a G_s G-protein, stimulating the activity of adenylylase and the production of cAMP [3–7]. If the activity of any of these proteins is inhibited, the follicle-enclosed oocyte is no longer able to maintain meiotic arrest.

The mechanisms that regulate meiotic arrest and resumption in the human oocyte are not as well understood due to the limited availability of material for study. However, the widespread use of in vitro fertilization (IVF) has provided an opportunity to obtain human oocytes for study. Results from the limited number of studies that have been done to date suggest that meiotic arrest may be regulated by a similar pathway as in rodents. For example, prophase I-stage human oocytes released from their follicles mature spontaneously in culture [8–10], and this can be reversibly inhibited by incubating oocytes in the presence of phosphodiesterase inhibitors [11, 12], demonstrating that cAMP is likely to have an important role in meiotic regulation. In addition, human oocytes contain the same cell cycle regulatory proteins that regulate meiosis in a diverse array of species [13, 14]. However, one important difference between humans and rodents is the length of their cycles. In humans, oocytes acquire meiotic competence and attain full size during the menstrual cycle, which generally lasts about 28 days, whereas rodent oocytes grow and acquire meiotic competence during the much shorter estrous cycle (typically about 4–5 days). The increased time during which meiotically competent oocytes must remain arrested in human oocytes compared to rodent oocytes could require additional mechanisms to keep oocytes arrested in prophase until the LH surge occurs. It is therefore important to examine if human oocyte meiotic arrest and resumption are regulated by similar mechanisms as in rodents.

In this study, we addressed the question of how meiotic arrest is maintained in human oocytes, using similar approaches to those used previously for studies of rodent oocytes. In particular, we examined whether human oocytes contain the same components of the signaling pathways leading to the production of cAMP as well as the requirement for G_s activity in the maintenance of meiotic arrest. Our results demonstrate that human oocytes maintain meiotic arrest prior to the LH surge using a signaling pathway similar to that of rodent oocytes.

MATERIALS AND METHODS

Source of Human and Mouse Oocytes

This study was approved by the Institutional Review Board at the University of Connecticut Health Center (IRB #06–125). Prior to participation

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²Correspondence: Lisa M. Mehlmann, Department of Cell Biology, University of Connecticut Health Center, Farmington, CT 06030. FAX: 860 679 1269; e-mail: lmehlman@neuron.uhc.edu

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in the study, all patients gave informed consent to donate immature oocytes. Immature oocytes were retrieved from ovaries of women ages 21–44 who were undergoing standard in vitro fertilization procedures using intracytoplasmic sperm injection. All patients underwent pituitary suppression with either a GnRH agonist or antagonist. Controlled ovarian stimulation was achieved with daily subcutaneous injections of 150–450 IU recombinant follicle-stimulating hormone with or without 75–150 IU human menopausal gonadotropin. Doses were adjusted based on follicular response as evidenced by serial transvaginal ultrasounds and serum estradiol levels. A subcutaneous injection of 3300–10 000 IU hCG was administered when three or more follicles reached a mean diameter of 18 mm. Transvaginal ultrasound-guided oocyte retrieval was performed 35 h after hCG injection.

Oocytes were aspirated from ovarian follicles approximately 14–22 mm in diameter. Most oocytes retrieved from such ovaries are at the metaphase II stage (mature eggs). Approximately 10%–15% of oocytes are in prophase I (immature); these oocytes are identified by the presence of the nucleus, or germinal vesicle (GV). As these immature oocytes are not clinically utilized at our center, they are routinely discarded. Oocyte-cumulus complexes were aspirated into culture medium (see the following) and incubated in an environment of 5% CO₂ and 95% air at 37°C for 3–5 h. Cumulus cells were stripped enzymatically using hyaluronidase type VIII from bovine testes (cat. #H-3757; Sigma-Aldrich, St. Louis, MO) and mechanically by being pipetted up and down through a small-bore pipet. Following cumulus removal, oocytes that had a GV were placed into medium containing 10 μM cilostamide (Calbiochem, La Jolla, CA), a phosphodiesterase 3A-specific inhibitor that prevents spontaneous meiotic resumption. Oocytes ranged in diameter from 105 to 115 μm. Oocytes retrieved from all patients on a given day were pooled.

Fully grown, GV-stage mouse oocytes were obtained from the ovaries of 22- to 25-day-old B6SJLF1 mice (Jackson Laboratory, Bar Harbor, ME) that had been primed with 5 IU eCG 40–46 h prior to collection. Cumulus cells were removed by repeated aspiration through a small-bore pipet. The medium for oocyte collection was HEPES-buffered minimal essential medium [15] supplemented with 250 μM dibutyryl cAMP to prevent spontaneous maturation during the collection process. All experiments were done with prior approval of the Animal Care and Use Committee at the University of Connecticut Health Center.

RT-PCR and Western Blotting

Germinal vesicle-stage human oocytes were placed in microcentrifuge tubes shortly after isolation from their cumulus complexes and were pelleted gently in small microfuge tubes. Most of the culture medium was removed, and oocytes were washed in PBS to remove serum, pelleted gently, frozen in liquid nitrogen, and stored at –80°C until use. For RT-PCR, mRNA was purified from 9 to 32 oocytes using TRIzol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. Any contaminating DNA was removed using the TURBO DNA-free Kit (#AM1907; Ambion, Austin, TX). Complementary DNA was prepared using the Superscript III Reverse Transcriptase Kit (Invitrogen) according to the manufacturer's instructions and using oligo-dT as the primer.

Following reverse transcription, 3.0–6.5 oocyte equivalents were used for PCR to amplify *GPR3*, *GPR12*, and type 3 adenylyl cyclase (*AC3*) using the following primer sequences: *GPR3*: 5'-CTCCACGGTTCAGAATGTT-3' and 5'-GGGAGAAGGCTCTGGTTTCT-3' (485-bp product); *GPR12*: 5'-GACGTACCATTCGGAGAGGA-3' and 5'-TGACAGGGTTGATGATGGAA-3' (461-bp product); *AC3*: 5'-GCTGGAATTGACTGGTGT-3' and 5'-GCGCACAGGTAGAGGAAGAC-3' (376-bp product). *GPR6* was amplified using the primer sequences 5'-TCCCATCATCTATGCCTCC-3' and 5'-TTTTGGCAGTTCAGCAGCTA-3' (496-bp product). Human ovary and brain cDNA were purchased from Ambion. The cycling parameters were an initial denaturation of 2 min, 94°C, followed by 40 cycles of 94°C for 30 sec, 55°C for 45 sec, 72°C for 45 sec, and a final extension of 72°C for 7 min. PCR products were electrophoresed on 1.5% agarose gels, the gels were visualized by staining with SYBRGold nucleic acid stain (cat. #S11494; Molecular Probes, Eugene, OR), and the products were photographed using Polaroid film (Rochester, NY). The identity of *GPR3* was confirmed by TA cloning (TA Cloning Kit, cat. #45-0046; Invitrogen) and sequencing, using T7 as the primer, and the resulting sequence compared with the human sequence published in GenBank.

Western blotting was performed as previously described [16]. Briefly, frozen oocytes were solubilized in Laemmli sample buffer and proteins separated on 10% acrylamide gels. G α_s was detected using an affinity purified antibody generated in rabbit against the C-terminal decapeptide of human G α_s (RM) [17]; these 10 amino acids are identical in human and mouse. This antibody, which was also used for microinjection (see the following), was generously provided by Teresa Jones of the National Institutes of Health.

Oocyte Culture

To determine what percentage of immature human oocytes underwent spontaneous maturation under our culture conditions, GV-stage oocytes were cultured in either Quinn Advantage Cleavage Medium (cat. #ART-1027; SAGE BioPharma, Bedminster, NJ) or in Quinn Advantage Blastocyst Medium (cat. #ART-1029; SAGE), supplemented with Quinn SPS Serum Protein Substitute (cat. #ART-3011; SAGE) and incubated in a humidified environment of 5% CO₂ and 95% air at 37°C. These media differ slightly in composition, with the major difference being that blastocyst medium contains several more amino acids than cleavage medium. Both types of media yielded the same results and herein will be referred to as culture medium.

To determine whether spontaneous maturation of GV-stage oocytes could be inhibited reversibly under our culture conditions, GV-stage oocytes were placed in culture medium containing 10 μM cilostamide immediately after the surrounding cumulus cells were removed with enzymatic and mechanical stripping. Following a 24–48 h culture period in a 200 μl drop of medium under light paraffin oil (Fisher Scientific, Pittsburgh, PA), GV-intact oocytes were washed into fresh medium without cilostamide and were observed using a Wild stereomicroscope for germinal vesicle breakdown (GVBD) and polar body formation.

Oocyte Microinjection

For microinjection experiments, GV-stage oocytes were placed in culture medium containing 10 μM cilostamide immediately after the surrounding cumulus cells were removed with hyaluronidase. Oocytes were cultured overnight, and oocytes containing intact GVs the following morning were used for microinjection. Microinjection was carried out as previously described [18]. Briefly, oocytes were quantitatively microinjected using a pressure system and pipets backfilled with mercury [18, 19]. The antibody was spin-dialyzed and concentrated in PBS as described previously [20]. The nonimmune rabbit IgG used for controls was purchased from Santa Cruz Biotechnology (Santa Cruz, CA) and was concentrated in the same way. Both the G α_s antibody and IgG stocks were diluted to 6 mg/ml, and 20–30 pl was injected to give a final intracellular concentration of 170–260 μg/ml in the oocyte. Both G α_s antibody-injected and IgG-injected oocytes were scored for GVBD periodically. Final concentrations in the oocytes were calculated based on an oocyte volume of 700 pl, which was calculated by averaging the volume of 105-μm and 115-μm diameter oocytes. Following microinjection, oocytes were placed in 200 μl drops of medium containing cilostamide, under oil, and were cultured in a humidified atmosphere at 37°C with 5% CO₂ and 95% air. Oocytes were scored periodically for the presence or absence of a GV.

RESULTS

Expression of GPR3 and Adenylyl Cyclase RNAs in Human Oocytes

In mouse oocytes, GPR3 is the receptor required for the oocyte to maintain meiotic arrest [3–5, 21], and the related receptor, GPR12, maintains meiotic arrest in the rat oocyte [5]. Due to the limited availability of human oocytes, we initially examined, using RT-PCR, which of these receptors, as well as the closely related receptor, *GPR6* [22], are expressed in the human ovary. Bands of the expected sizes were amplified for both *GPR3* and *GPR12* (Fig. 1). *GPR6* was not expressed, although the primers used detected a specific band in human brain.

Therefore, we examined if *GPR3* and/or *GPR12* were expressed in GV-stage human oocytes. A band of the expected size for *GPR3* was amplified from cDNA derived from oocytes (Fig. 2), and its identity was confirmed by cloning and sequencing the PCR product. *GPR12* was not detectable using the same cycling parameters as for *GPR3* (Fig. 2 and legend), demonstrating that *GPR3* is the only member of the *GPR3/GPR6/GPR12* family detectable in the human oocyte. No band was detected when PCR was performed on RNA that had not undergone reverse transcription using the *GPR3* primer set, demonstrating that there was no significant genomic DNA contamination (Fig. 2). In addition, the absence of a band for *GPR12*, despite a functional primer set, shows that there was no genomic DNA contamination.

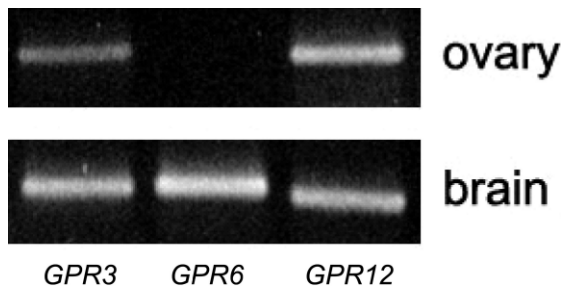


FIG. 1. The G_s-coupled receptors, *GPR3* and *GPR12*, but not *GPR6*, are expressed in human ovary. Upper panel: RT-PCR demonstrating the presence of *GPR3* and *GPR12* RNAs in human ovary. *GPR6* is not detectable, though it was amplified from human brain cDNA, demonstrating that the primers were functional (lower panel). We used 1 ng of each cDNA in these experiments, which were performed once using ovary and twice using brain.

In addition to *GPR3*, *GPR6*, and *GPR12*, we examined whether *AC3*, which is necessary for the maintenance of meiotic arrest in mouse oocytes [23], is expressed in human oocytes. A band of the expected size for *AC3* was amplified from cDNA derived from human ovary as well as in GV-stage oocytes (Fig. 3).

Expression of G_s Protein in Human Oocytes

We also examined the presence of the G_s G-protein in GV-stage human oocytes, using Western blot analysis. Mouse oocytes contain two forms of G_s, a long form and a short form [6], and this protein is expressed in a high abundance, such that it can be detected using a small number of oocytes (Fig. 4B). Likewise, both forms of G_s were specifically detected in human oocytes (Fig. 4). Comparing the intensity of the bands in mouse and human oocytes, the amount of G_s appears to be similar (Fig. 4B and legend). Taken together, the expression of *GPR3* and *AC3* mRNA as well as abundant G_s protein present in human oocytes suggest that meiotic arrest could be regulated by the same pathway as in the mouse.

Establishing Culture Conditions to Control Meiotic Arrest and Resumption in Human Oocytes

Previous studies have shown that GV-stage human oocytes mature spontaneously in culture when removed from the follicle [8, 24–26] (Fig. 5), and this spontaneous maturation can be reversibly inhibited by phosphodiesterase inhibitors [11, 12]. However, the timing of GVBD following phosphodiesterase

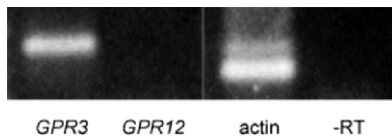


FIG. 2. *GPR3* RNA is expressed in human oocytes. RT-PCR using cDNA derived from 6.5 human oocyte equivalents amplified bands for *GPR3*, whereas *GPR12* was not detected. All of the PCR products were performed on the same cDNA and run on the same gel. The presence of *GPR3*, as well as actin, demonstrates the quality of the cDNA. We ruled out possible genomic contamination by performing PCR on the same batch of RNA that had not undergone reverse transcription (-RT). We estimate that the amount of cDNA used for these PCR reactions corresponded to approximately 20 ng total RNA. This figure is based on the amount of RNA in a mouse oocyte (approximately 1 ng) [3] and taking into account that human oocytes contain three times the volume of mouse oocytes. Each experiment was performed seven times or more, using at least six different batches of cDNA.

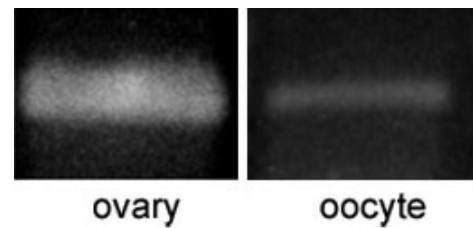


FIG. 3. Expression of *AC3* RNA in human ovary and oocytes. RT-PCR using cDNA derived from five human oocytes (approximately 15 ng RNA) and 1 ng cDNA (ovary). Experiments were performed once using ovary and twice using oocytes.

inhibitor removal has not been carefully characterized. As a prelude to experiments to examine whether the G_s signaling pathway is involved in maintaining meiotic arrest in human oocytes, we determined the percentage of GV-stage oocytes that matured spontaneously under our culture conditions, as well as the timing of GVBD following removal of the phosphodiesterase inhibitor, cilostamide.

Figure 5A shows two human oocytes that were GV-intact at the time of retrieval and matured in culture to the metaphase II stage, undergoing GVBD and first polar body extrusion within 24 h after oocyte retrieval. The phosphodiesterase type 3A (PDE3A)-specific inhibitor, cilostamide (10 μM), prevented spontaneous meiotic resumption in 94% of oocytes that had readily discernible GVs (examined using a stereomicroscope) at the time that they were placed in culture (n = 36); meiotic arrest was maintained in these oocytes during overnight culture. Seventy-one percent of these GV-stage oocytes underwent GVBD within 24 h after they were subsequently transferred to culture medium without cilostamide, demonstrating that these oocytes were competent and that the meiotic arrest was reversible. The time from cilostamide removal to GVBD was variable, ranging from 2 to 48 h (Fig. 5B); for these studies, oocytes that underwent GVBD within 24 h were considered to be competent. These results demonstrate that with our culture conditions, we can maintain meiotic arrest in human oocytes

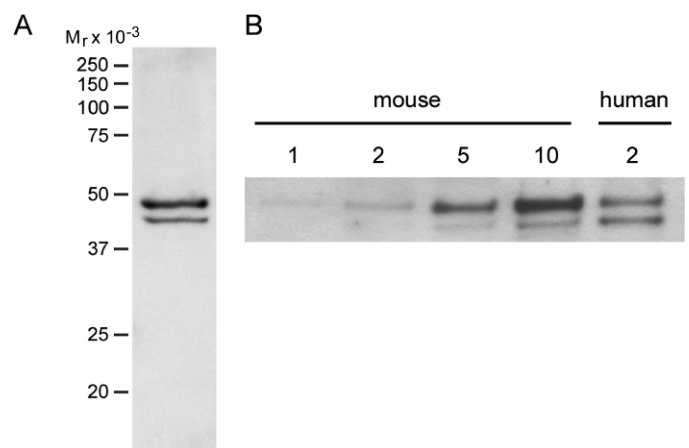


FIG. 4. Human oocytes contain the G_s G-protein. **A**) Western blot showing specific labeling of both the long and short forms of G_s protein in lysates from 13 human oocytes (approximately 1.5 μg). **B**) The amount of G_s protein is comparable in mouse and human oocytes. Because human oocytes have three times the volume of mouse oocytes, and because the intensities of the G_s bands in five mouse oocytes and two human oocytes are comparable, the concentration of G_s protein in human and mouse oocytes is similar. Number of oocytes is indicated above each lane. Human oocyte blots were performed five times.

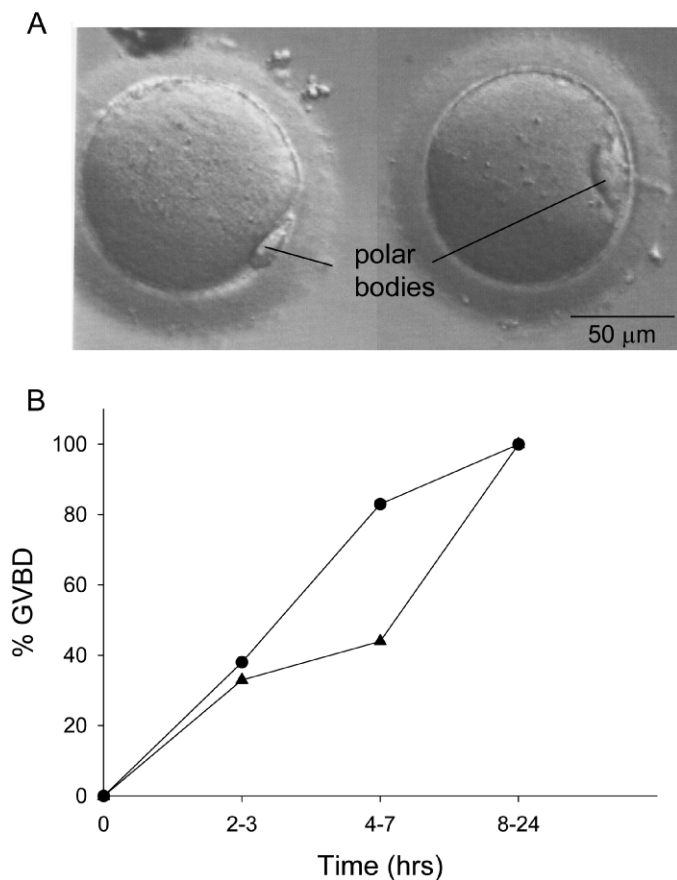


FIG. 5. In vitro maturation of human oocytes. **A**) GV-stage oocytes retrieved from a patient undergoing IVF matured spontaneously in culture, undergoing GVBD and first polar body formation. Bar = 50 μ m. **B**) Time course of spontaneous meiotic resumption. GV-stage oocytes were maintained in meiotic arrest by incubation overnight in medium containing 10 μ M cilostamide. Oocytes were scored for GVBD after being washed out of the cilostamide. Circles, time course of GVBD in 24 uninjected oocytes; triangles, time course of GVBD in oocytes that resumed meiosis in response to injection of a G_s antibody in the presence of 10 μ M cilostamide (see Fig. 6).

and control the onset of meiotic resumption. They also provide a basis for examining the requirement for the G_s signaling pathway in maintaining meiotic arrest.

Activity of the G_s G-Protein is Required for Maintenance of Meiotic Arrest in Human Oocytes

To examine if G_s activity is necessary to maintain oocytes in prophase arrest, we determined if injection of an antibody against $G\alpha_s$ could overcome the meiotic inhibition of oocytes cultured in the presence of 10 μ M cilostamide. The affinity purified antibody we used has previously been shown to inhibit G_s activity [17, 20] and causes GVBD when injected into frog and mouse oocytes [6, 20]. The specificity of this antibody for human $G\alpha_s$, shown in Figure 4, establishes that it is an appropriate antibody to use for these microinjection experiments. We injected a total of 22 oocytes that contained well-defined GVs following a 19–25 h culture in medium containing cilostamide with 170–260 μ g/ml of the G_s antibody or nonimmune IgG. Following microinjection, oocytes were cultured in the presence of cilostamide and 5% CO_2 , 95% air, and examined periodically for the presence or absence of the GV.

Ninety percent of the oocytes injected with the G_s antibody underwent GVBD within 2–19 h following antibody injection

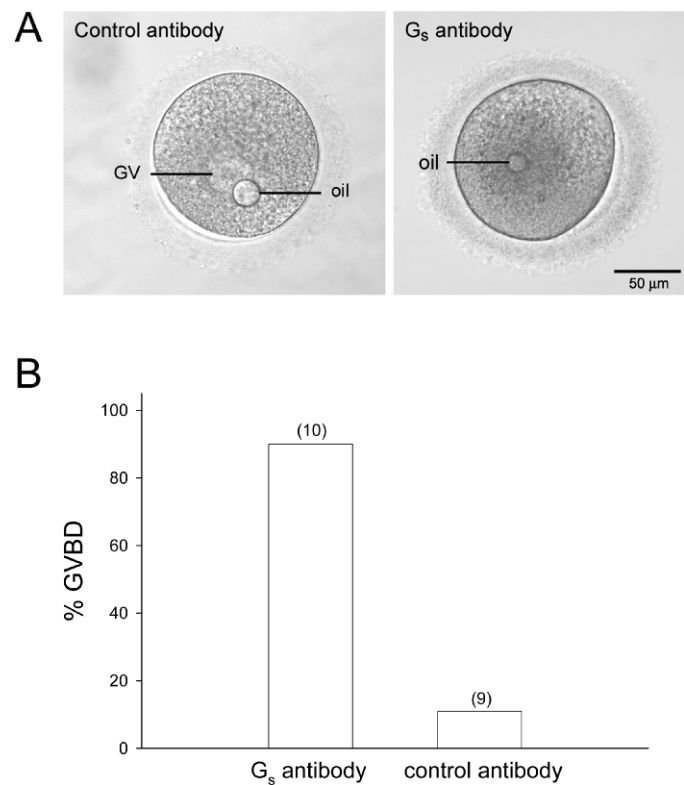


FIG. 6. Inhibiting the activity of G_s causes meiotic resumption in human oocytes. **A**) Oocytes microinjected with 170–260 μ g/ml G_s antibody or nonimmune IgG in the presence of, and following an overnight culture in, 10 μ M cilostamide. Oocytes injected with the G_s antibody (right) underwent GVBD, whereas control IgG-injected oocytes (left) remained arrested at the GV stage. The oil drops were introduced into the oocytes as a consequence of the microinjection procedure. **B**) Percentages of oocytes that underwent GVBD following injection of the G_s antibody or control IgG. The numbers in parentheses are total number of oocytes injected.

($n = 10$; Figs. 5B, 6B and legend). Of these, 70% formed first polar bodies after further culture, suggesting that the resumption of meiosis was physiological and that the injected oocytes were healthy. Eighty-nine percent of oocytes injected with nonimmune IgG and maintained in the presence of cilostamide ($n = 9$) did not undergo GVBD during the culture period but underwent GVBD after they were washed out of cilostamide (Fig. 6B). Only meiotically competent oocytes were included in this study; oocytes were considered to be competent if they underwent GVBD within 24 h after they were washed out of cilostamide. Eighty-three percent (10/12) of the oocytes injected with the G_s antibody were competent, and 90% (9/10) of the oocytes injected with IgG were competent. These percentages were similar to those we obtained from uninjected oocytes that were washed out of cilostamide following overnight culture. Oocytes injected with control IgG were cultured for either 6 ($n = 2$) or 19–21 h ($n = 8$) prior to being washed out of the cilostamide; these oocytes were pooled for this analysis ($n = 10$ total).

DISCUSSION

Recent results from several studies have shown that the G_s signal transduction pathway is necessary to maintain meiotic arrest in rodent oocytes, prior to the surge of LH that stimulates meiotic resumption. This signaling pathway is initiated by a constitutively active G-protein coupled receptor, GPR3 (mice) [3–6, 21] or GPR12 (rats) [5] in the oocyte. In the present

study, we show that the G_s signaling pathway is also required to maintain meiotic arrest in the human oocyte. We found that human oocytes express RNAs encoding GPR3 and AC3 and express G_{α_s} protein. Due to the difficulty of detecting native levels of G-protein-coupled receptor protein using antibodies [27] as well as the inability to detect native levels of GPR3 in mouse oocytes [21], we were unable to examine if GPR3 protein is also present in the human oocyte.

The related receptors, *GPR12* and *GPR6*, are not expressed in human oocytes (Figs. 1 and 2). Previous studies by others have shown that GV-stage human oocytes remain arrested at prophase I when cultured with PDE3 inhibitors [11, 12], indicating further that cAMP in the human oocyte is important for regulating meiosis. Here, we have shown that inhibiting G_s activity using a function-blocking antibody caused isolated oocytes to mature spontaneously when cultured in the presence of the PDE3A-specific inhibitor cilostamide. These results indicate that in human oocytes, as in their rodent counterparts, cAMP levels regulate meiotic arrest and that the G_s signaling pathway is necessary for maintenance of meiotic arrest.

The somatic cells surrounding the mammalian oocyte are also necessary for the maintenance of meiotic arrest prior to the LH surge, as reviewed in Eppig et al. [13]. It is not yet known what role the somatic cells have in this process. In mice, as well as in larger mammals such as cows and pigs, the cumulus cells alone are not sufficient to hold the oocyte in prophase I arrest, whereas meiotic arrest is maintained in cumulus-enclosed oocytes that are physically connected to the surrounding mural granulosa cells [28–32]. Both isolated as well as cumulus-enclosed mouse oocytes resume meiosis soon after a fall in cAMP levels following isolation from the follicle [28, 33] in the absence of PDE inhibitor, although there is some evidence that the presence of the cumulus cells delays meiotic resumption slightly [28].

In contrast, we found that meiotic prophase I arrest could be maintained in cumulus-enclosed human oocytes for several hours prior to the isolated oocytes being placed in cilostamide. This difference could be due to a slower degradation of cAMP in human oocytes compared to rodents or to another inhibitory factor from the cumulus cells. It is interesting to note that *GPR12* is expressed in the human ovary and could conceivably provide a source of cAMP to the oocyte through gap junctions. A role for the cumulus cells in maintaining human meiotic arrest needs to be investigated further. Nevertheless, the finding that human oocyte meiotic arrest is regulated by a G_s pathway suggests that rodent models can be useful for studies of human oocyte maturation. In addition, the methods used here demonstrate that we can inhibit meiotic resumption in GV-stage human oocytes and that we can experimentally stimulate meiotic resumption by targeting components of the cell signaling pathways responsible for meiotic resumption.

A limitation of this study is that the GV-stage oocytes available to us were retrieved from hormonally stimulated women, and the follicles they were retrieved from may have been too small to express a sufficient number of LH receptors to respond to the LH surge. However, for several reasons, these oocytes are not necessarily inappropriate for this study. In mice, the GPR3/G_s signaling pathway is in place and functional prior to the acquisition of meiotic competence [7]. In humans, meiotic competence is acquired when follicles are between 5 and 7 mm in diameter [25], whereas the GV-stage oocytes used in this study were retrieved from follicles at least 14 mm in diameter. In addition, these oocytes underwent GVBD and formed polar bodies in culture, demonstrating that they were competent. Furthermore, some in vitro matured oocytes that were obtained in this way have been fertilized and produced

live offspring [26], demonstrating that they can be physiologically normal. The developmental competence of oocytes obtained during this stage is low, however, even in oocytes retrieved from women who have not been hormonally stimulated [34–36]. Further studies are needed to identify the mechanisms by which human meiosis is regulated in oocytes as well as the normal intracellular events that occur during human oocyte maturation in order to accurately assess the quality of oocytes matured in vitro.

A more complete understanding of the cellular processes involved in human oocyte maturation could potentially lead to improved culture conditions for maturing human oocytes in vitro (in vitro maturation; IVM). IVM has become a topic of intense research because the ability to mature human oocytes in vitro could significantly improve methods for infertility treatment. Currently, standard IVF protocols require hormone injections, the inconvenience of frequent monitoring of follicular response, unpleasant side effects, and the potential risk of developing ovarian hyperstimulation syndrome, an often dangerous medical condition that may necessitate cycle cancellation and hospitalization in severe cases [37, 38]. With IVM, women could potentially undergo IVF with a significant reduction in these risks. In addition, IVM could provide some women the opportunity to preserve their fertility, particularly female cancer patients who are at risk of becoming infertile as a result of treatment [39–41]. Despite improving success rates, IVM is still in its infancy, and only about 400–500 babies have been born worldwide from this procedure [10]. Results from the limited number of studies examining the basic science of human IVM have shown that several aspects of nuclear and cytoplasmic maturation are abnormal in these oocytes [11, 14, 42–44]. All of these factors are likely to contribute to the low developmental potential of fertilized, in vitro matured oocytes.

With an improved understanding of the mechanisms that regulate meiotic competence, arrest, and resumption, we potentially could culture human oocytes under conditions that more closely simulate their physiological environment, and this, in turn, could improve the quality of mature oocytes retrieved following IVM. For example, immature oocytes that are cultured in the presence of the cumulus cells that normally surround the oocyte, as well as those cultured in a gel matrix that more accurately simulates the three-dimensional environment of the oocyte-cumulus cell complex, have improved cytoplasmic maturation rates compared with oocytes separated from the cumulus cells [14, 45]. By identifying the importance of the GPR3/G_s signaling pathway in human oocytes, we add another component to our understanding of an important stage of meiotic regulation. In combination with a better understanding of the role for the cumulus cells in this process, methods for culturing cumulus-enclosed oocytes could potentially be improved such that these oocytes could be cultured under conditions that more accurately simulate their native environment. More studies are needed to clarify the physiological events that occur during normal nuclear and cytoplasmic maturation as well as to compare the quality of oocytes matured from small follicles of hormonally unstimulated women with those obtained during standard, hormonally stimulated IVF cycles.

In summary, we report new aspects of the signaling pathway in the human oocyte that is necessary to maintain meiotic arrest prior to the LH surge. We also have shown that human meiotic maturation can be studied in a controlled way. This can be expanded to future studies examining the mechanisms of meiotic maintenance and resumption in human oocytes.

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