Primate preimplantation embryo is a target for relaxin during early pregnancy

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Objective: To determine whether preimplantation embryos are targets for relaxin secreted from the corpus luteum of the menstrual cycle.
Design: Rhesus monkey oocytes obtained from females undergoing controlled ovarian hyperstimulation were inseminated, and the resulting embryos were cultured in medium with or without recombinant human relaxin (20 ng/mL) for 8 days.
Setting: Research laboratory.
Animal(s): Rhesus monkey.
Intervention(s): Controlled ovarian stimulation to obtain oocytes for in vitro–produced embryos that were cultured with or without human recombinant relaxin.
Main Outcome Measure(s): Rate of blastocyst development, percentage of blastocysts, and inner cell mass/trophectoderm cell ratio were measured on day 8 of culture. The presence of relaxin receptor (*RXFP1*) messenger RNA in eight-cell embryos was observed by array hybridization.
Result(s): *RXFP1* receptor expression was localized to the inner cell mass of blastocysts, as shown by immunohistochemistry. The percentage of embryos that developed to blastocyst and the inner cell mass/trophectoderm cell ratio was unchanged with relaxin supplementation; however, the relaxin-treated embryos developed into blastocysts significantly sooner than untreated embryos.
Conclusion(s): These results are the first evidence that the preimplantation primate embryo is a target for relaxin and that the addition of relaxin to in vitro culture medium enhances rhesus monkey embryo development. (Fertil Steril® 2011;96:203–7. ©2011 by American Society for Reproductive Medicine.)

Key Words: Gene expression, granulosa cells, blastocyst, rhesus macaque

Since first being described in the 1920s (1), relaxin has been associated with pregnancy and parturition in many mammalian species (2). In human and nonhuman primates, circulating relaxin is seen during the luteal phase of the menstrual cycle and during early pregnancy, and as early as day 7 after ovulation in humans (3, 4) and monkeys (5). It is secreted by human luteinizing granulosa cells as early as day 5 after aspiration during IVF cycles (6). Relaxin is also produced by the deciduae and trophoblast (7). Although Yki-Jarvinen et al. (8) did not detect relaxin in the oviduct; Tang and Chegini (9) reported relaxin gene expression by reverse transcription–polymerase chain reaction with immunohistochemical localization in tubal epithelium of the ampullary and isthmus regions.

Relaxin binds to two receptors that modulate cyclic adenosine monophosphate production: LGR7 and LGR8 (10). These were later renamed RXFP1 and RXFP2 (11), with RXFP1 recognized as the primary receptor for relaxin and RXFP2 as the primary receptor for INSL3 (12). Receptors for relaxin have been localized to a variety of reproductive and nonreproductive tissues by messenger RNA (mRNA) or protein expression (12).

The roles of relaxin during the pre- and peri-implantation period remain unclear, but relaxin is known to target the endometrium during the peri-implantation period. A peri-implantation effect of relaxin has been known for some time in the mouse (13), but a recent study in the marmoset found that relaxin and uterine RXFP1 were up-regulated during this period (14). Relaxin stimulates decidual cell production of IGFBP1, PRL secretion from decidual and stromal cells, secretion of glycodelin, and vascular endothelial growth factor secretion (15, 16). Relaxin may also affect the ovary, which expresses relaxin receptors (17). Another possible target for relaxin would be the embryo itself. The timing of luteal relaxin production indicates that it is available to blastocysts and peri-implantation embryo. However, neither the detection of relaxin receptors in blastocysts or peri-implantion embryos nor the effects of H2 relaxin on blastocyst development have been reported. Here we have used the rhesus monkey model to determine whether preimplantation embryos may be targets for relaxin secreted during the peri-implantation period.

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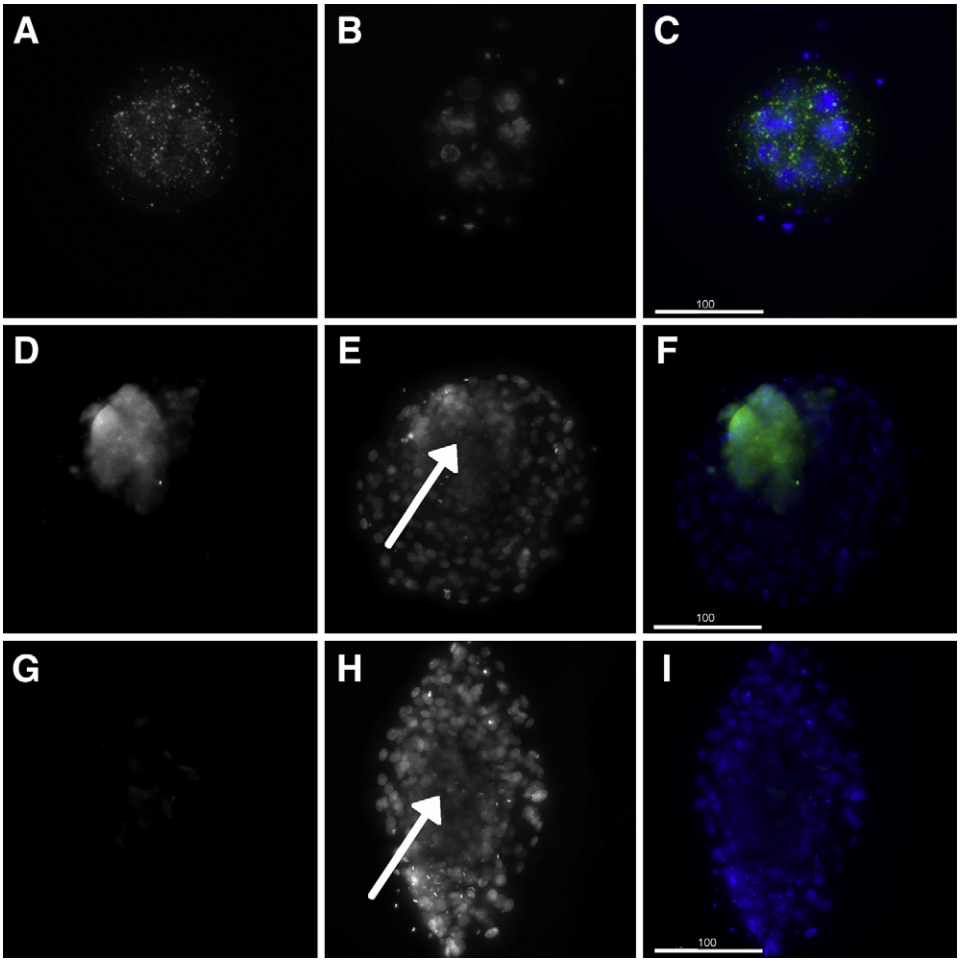
MATERIALS AND METHODS

Animal Husbandry

Adult female rhesus macaques (*Macaca mulatta*) were housed at the California National Primate Research Center as previously described (18). Only

FIGURE 1

Relaxin receptor antibody staining of monkey eight-celled embryo and blastocysts. (**A–C**) Eight-celled embryo. (**D–I**) Blastocysts. Original magnification, ×190. All are whole reconstructed embryos. Scale bars = 100 μm. (**A, D, G**) RXFP1 antibody staining, with **G** showing lack of labeling for the mouse IgG control. (**B, E, H**) DAPI labeling for embryos, with arrows in **E** and **H** indicating the approximate center of the ICM. (**C, F, I**) Merged images of **A** and **B**, **D** and **E**, and **G** and **H**, respectively.



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females with a history of normal menstrual cycles were selected for this study. All procedures for maintenance and handling of the animals were reviewed and approved in advance by the Institutional Animal Use and Care Administrative Advisory Committee at the University of California at Davis.

Females were observed daily for signs of vaginal bleeding, and the first day of menses was designated cycle day 1. Beginning on cycle day 1–4 recombinant human FSH (Organon) was administered (37.5 IU) twice daily, IM for 7 days total. To obtain in vivo–matured oocytes, females were given recombinant hCG (1,000 IU Ovidrel; Serono) on treatment day 8 in addition to the FSH treatment outlined above. Cumulus–oocyte complexes were removed at 28–30 h after hCG administration by ultrasound-guided aspiration (19, 20). Oocytes were retrieved from aspirates as previously described (18). Granulosa cells were obtained from the aspirates and immediately placed in buffer and stored frozen for later processing for gene expression.

IVF and Embryo Development

In vivo–matured oocytes were rinsed and transferred into Tyrode's lactate with polyvinyl alcohol medium (37°C) under oil and inseminated according to standard procedure for IVF of rhesus macaque oocytes (20); this was day 0 of embryo culture. Semen was collected from male macaques that had been trained for this procedure as previously described (21). Sperm were washed from seminal plasma and resuspended in Tyrode's lactate with bovine serum albumin medium (22). The next morning (day 1), oocytes were transferred into 70-μL drops of chemically defined, protein-free hamster embryo culture medium 9 (HECM-9) without or with relaxin (see below) under oil (37°C) and incubated at 37°C in a humidified atmosphere of 5% CO_2, 10% O_2, and 85% N_2 for 48 hours (23). At approximately 60 hours after insemination noncleaved oocytes were again assessed for developmental status. Noncleaved oocytes exhibiting a polar body and all embryos were classified as having matured to metaphase II of meiosis. Embryos were transferred into 70-μL drops of HECM-9 medium with 5% bovine calf serum (Gemini Bioproducts) with or without relaxin (see below) under mineral oil and incubated as described above. Embryos were transferred to fresh medium every other day until fixed on day 8 after insemination. Beginning on day 5, embryos were observed with a Nikon SMZ-2B microscope housed in a temperature-controlled isolette daily at 7:00 AM and 5:00 PM for developmental stage. On day 8, the percentage of embryos developing to the blastocyst stage was calculated for each treatment. The percentage blastocyst development for each female was then averaged to obtain the mean for each treatment.

Blastocyst-stage embryos were fixed and stained for differential cell counting with POU5F1 (also known as *Oct3/4*) as previously described (18).