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Ovarian Hyperstimulation Inhibits Embryo Implantation in the Mouse

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Embryo implantation is dependent on the synchronous development of the embryo and of the endometrium. Pharmacologic doses of estrogens change endometrial histology and are known to inhibit implantation. During controlled ovarian hyperstimulation, such as occurs during an in vitro fertilization cycle, serum estradiol levels may be elevated to as much as three to six times those found during spontaneous cycles. Serum progesterone levels are also increased and may counteract the elevated estradiol levels. The overall effect of ovarian stimulation on implantation is therefore not known. To study this question, we developed a mouse embryo donation model. Donor embryos were obtained in the late morula to early blastocyst stage from hyperstimulated mated mice. The donated embryos were then transferred to the uteri of two groups of recipient mice. The study group underwent ovarian hyperstimulation with pregnant mare's serum gonadotropin (PMSG) and human chorionic gonadotropin (hCG) (OHR group), while the controls were allowed to cycle spontaneously (SR group). All recipient mice underwent cervical stimulation to induce a pseudopregnant state. Five embryos were transferred to the left uterine horn of each of nine OHR mice and seven SR mice. A higher implantation rate was noted in the SR group than in the OHR group (50 ± 12 vs 8 ± 4%, $P < 0.001$). Our data suggest that, in the mouse, ovarian hyperstimulation impedes implantation by causing adverse changes in uterine receptivity.

KEY WORDS: mouse; blastocyst; implantation; hyperstimulation.

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INTRODUCTION

Pregnancy rates during in vitro fertilization (IVF) increase significantly when multiple embryos are transferred to the uterus after oocyte collection and embryo culture (1,2). In order to increase the number of embryos available for transfer, controlled ovarian hyperstimulation (OH) with exogenous gonadotropin therapy is used to increase the number of mature oocytes produced per cycle (3,4). Of the oocytes collected, most fertilize and undergo cleavage prior to embryo transfer. Yet no more than 10% of the embryos transferred actually implant and result in clinical pregnancies (5). This low implantation rate is thought to be caused by a combination of low embryo quality and, possibly, low endometrial receptivity.

Endometrial receptivity is known to be sensitive to changes in the serum levels of the sex steroids. Pharmacologic doses of estrogens may be used as interceptives to prevent embryo implantation after fertilization has taken place (6). This finding may apply to embryo implantation during IVF cycles, as ovarian hyperstimulation may elevate the serum estradiol (E_2) levels to as much as three to six times of those found during spontaneous cycles (7–10). These supraphysiologic levels of E_2 may impede implantation and thus lower pregnancy rates. The overall picture is more complex, however, as levels of other steroids, notably progesterone, are also elevated. While ovarian hyperstimulation has been shown to advance endometrial maturation as evaluated by the Noyes criteria (11), the clinical impact of ovarian hyperstimulation upon endometrial receptivity has not yet been measured. We therefore designed our study to compare embryo implantation rates during spontaneous and ovarian hyperstimulation cycles. In order to control for embryo quality, we chose a mouse embryo donation model.

MATERIALS AND METHODS

Donor Zygote Preparation

Twenty-six normal female C6B3F1 mice, 49–56 days old, underwent ovarian hyperstimulation with an intraperitoneal injection of 10 IU of pregnant mare's serum gonadotropin (PMSG; Sigma, St. Louis, Missouri), followed 48 hr later by 10 IU of human chorionic gonadotropin (hCG; Sigma). The females were then caged overnight with normal fertile C6B3F1 male mice. Ninety-two hours post-hCG, the mice were sacrificed, the uteri were removed, and each uterine horn was flushed with 0.3 ml of equilbrated Ham's F-10 medium. All embryos were then transferred to fresh medium in a single dish and inspected for viability. Intact (nonfragmenting) embryos in the late morula to early blastocyst stage were considered for transfer.

Ovarian Hyperstimulated Recipients (OHR)

In this group, ovarian hyperstimulation with the same regimen was accomplished synchronously with that of the donors as described above. Nine normal female C6B3F1 mice were used. At the time of the hCG intraperitoneal injection (time 0), as well as 2, 16, and 20 hr post-hCG, the cervix was stimulated using a blunt-tipped metal engraver for 10 sec to induce pseudopregnancy (12). Vaginal smears were performed 24 and 72 hr post-hCG. These mice were not housed with male mice.

Spontaneous Recipients (SR)

Vaginal smears were performed on an additional 94 spontaneously cycling C6B3F1 female mice at the time of the donor hCG injections described above. Thirty-seven mice were found to have a vaginal smear compatible with estrus. These mice underwent cervical stimulation at the same time as the OHR mice, i.e., at time = 0, 2, 16, and 20 hr as measured from the hCG injection in the donors. Vaginal smears were performed 24 and 72 hr post initiation of cervical stimulation as above. These mice were also not housed with male mice.

Embryo Transfer

All OHR and SR recipients, determined by vaginal smear to be in pseudopregnancy, were anesthesized with a combination of ketamine and metafluorane. A 1-cm left flank incision was made, and the peritoneum over the ovary opened (12). The left uterine horn was delivered through the incision, and a 25-gauge needle utilized to create an opening in an avascular space of the uterine horn. The endometrial cavity was thus reached and the needle withdrawn. Five nonfragmented embryos, suspended in 3–5 μl of medium, were then transferred with a glass pipette into the uterus. The uterus was replaced in the peritoneum, and the skin incision closed with 4-0 silk. Vaginal smears were checked for persistence of pseudopregnancy 2 and 5 days after transfer (12).

Observation of Implantation

All mice were sacrificed 5 days after embryo transfer. Uteri were removed and opened. Implantation sites were now clearly evident and were counted for each group. Table I summarizes the protocol.

Statistics

The Mann-Whitney non-paired test was used to compare the number of implantations in each group.

RESULTS

Twenty-six mice were used as donors and produced 143 intact embryos in the late morula to early blastocyst stage. Estrus was found by vaginal smear in 37% of the normally cycling mice. Twenty-four hours after cervical stimulation, 36 of the 37 SR mice and all of the 9 OHR mice were found to be in pseudopregnancy. Seventy-two hours after cervical stimulation, 7 of 37 SR mice and 9 of 9 OHR mice continued to be in pseudopregnancy (Table II). Ninety-two hours post-hCG, five embryos were

Table I. Protocol

Day	Embryo donor	Hyperstimulated recipient	Spontaneous recipient
1	10 IU PMSG	10 IU PMSG	—
3	10 IU hCG Mated	10 IU hCG Cx Stim	Vag Smear Cx Stim
4	—	Vag smear	Vag smear
6	Collect embryos	Vag smear, embryo transfer	Vag smear, embryo transfer
8	—	Vag Smear	Vag Smear
11	—	Vag Smear, count implantations	Vag smear, count implantations

therefore transferred to the left uterine horn of each pseudopregnant recipient. Thirty-five embryos were transferred to the seven pseudopregnant SR mice and 45 embryos were transferred to the nine pseudopregnant OHR mice.

Since pseudopregnancy is thought to be dependent on cervical stimulation only and not on ovum viability or embryo transfer (12), only those mice continuing to be in pseudopregnancy were included in the subsequent analysis.

Forty-eight hours after embryo transfer, four of the seven SR mice and all nine of the OHR mice were found to have vaginal smears consistent with persistant pseudopregnancy. Five days after embryo transfer, all of the recipient mice were sacrificed. All four of the SR mice continued to be in pseudopregnancy and a total of 10 implantations was counted in this group. At least two implantations were found in the left uterine horn of each SR mouse. In the OHR group, eight of the nine mice were in pseudopregnancy. One implantation was found in the left uterine horn of three of these mice, for a total of three implanting embryos (Table III). There were 2.5 ± 0.3 (mean ± SE) implantations per mouse in the SR group and 0.4 ± 0.2 implantations in the OHR group. This difference was statistically significant ($P < 0.001$).

DISCUSSION

The mouse estrous cycle consists of four phases—proestrus, estrus, metestrus, and diestrus—and is roughly analogous to the follicular phase of the human cycle (12). In the absence of cervical stimulation the corpus luteum secretes little progesterone, is functional for a short period of time, and will not support pregnancy (5). Cervical stimulation during the estrous phase of the cycle, if successful, activates the corpus luteum for 14 days (5). This is then described as pseudopregnancy. A typical progestational state occurs, which is characterized by elevated levels of progesterone and endometrial changes that aid implantation. These changes are analogous to the luteal phase of the menstrual cycle. Cervical stimulation either through mating in the donor group or artificially in the recipient groups was induced simultaneously. The rise in progesterone would therefore be expected to occur concurrently in all groups. Therefore, the only difference between the recipient groups should be ovarian hyperstimulation in the OHR group.

Table II. Vaginal Smear Results: Number of mice in Pseudopregnancy

	Day of experiment				
Recipient type	3	4	6 (ET)	8	11
Spontaneous	37	36	7	7	4
Hyperstimulated	14	14	9	9	8

Table III. Embryo Implantations

Recipient Type	Number of mice	Embryos transferred	Number of implantations	Percent implantations
Spontaneous	4	20	10*	50%
Hyperstimulated	8	40	3*	8%

* $p < 0.05$

The implantation rate of 50% per embryo transferred found in the spontaneous group was similar to that found by other investigators when standard embryo transfer techniques were used (5,13–19). The embryos were randomly distributed among the SR and OHR groups. The timing and the technique of embryo transfer were identical in the two groups. The implantation rate of 8% per embryo transferred found in the OHR group is significantly lower than in the SR group ($P < 0.001$).

Successful implantation of the developing embryo is dependent on the receptivity of the endometrium, which in turn, is controlled by the gonadal sex steroids. Estradiol induces mitotic activity in the stromal and glandular tissues, leading to an increase in the height of the endometrium and an increase in uterine blood flow (13,20). Luteal-phase progesterone inhibits mitotic activity and induces glandular secretion (20). An early rise or prolonged elevation of the preovulatory levels of estrogen has been shown to lead to decreased pregnancy rates and increased pre- and postimplantation death of the embryo (5). The administration of high-dose estrogen in the postovulatory period is effective in preventing pregnancy (6). Therefore, it is of some concern that ovarian hyperstimulation, while increasing the number of available oocytes, also causes serum E_2 levels to be elevated and may therefore impede implantation.

While progesterone levels have also been found to be elevated during ovarian hyperstimulation, high progesterone levels alone are not inhibitory to implantation (5) and may help reverse the adverse affects of high E_2 (11). In the mouse, the adminis-

tration of progesterone alone in doses of 1 to 10 mg did not interfere with embryo implantation (5). While the administration of 1–10 mg of E_2 did significantly inhibit implantation, this effect could be reversed with the administration of progesterone in the same high dose (5). These data suggest that the inhibitory effects of the high serum E_2 levels found during ovarian hyperstimulation may therefore be at least partly counteracted by the high progesterone levels associated with this state.

The morphology of the endometrium after ovarian hyperstimulation has been studied previously (11,21). Light microscopy has shown advanced endometrial maturation after ovulation induction with human menopausal gonadotropin (hMG)/hCG (11). In clomiphene citrate cycles, a consistent difference in endometrial histology has not been shown when biopsies were examined with scanning electron microscopy (21). However, the antiestrogenic effect of clomiphene citrate may mask the effects of the elevated E_2 on the endometrium. Therefore, scanning electron microscopy may be able to detect the influence of elevated E_2 levels when hMG is used alone and not when clomiphene citrate is used alone or in conjunction with hMG.

One of the ways in which elevated E_2 levels may adversely affect the endometrium may be related to altering the synchrony of the development of the embryos and of the endometrium. An embryo more than 2 days further developed than the endometrium will not implant (15). Similarly, endometrium more than 1 day further advanced than the embryo will not allow implantation (15). Elevated levels of estradiol may slow the maturation of the endometrium and thus decrease implantation rates (14). The endometrium in the OHR group may therefore have been less mature than that in the spontaneous group. The elevated levels of E_2 found during ovarian hyperstimulation would then result in lower implantation rates, as we found.

These data therefore suggest that ovarian hyperstimulation significantly impedes implantation in the mouse.

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