Metoclopramide-induced hyperprolactinaemia caused marked decline in pinopodes and pregnancy rates in mice

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BACKGROUND: The impact of hyperprolactinaemia on endometrial function, along with embryo implantation, has been the subject of discussion. This article examines whether experimental hyperprolactinaemia can affect mouse ovarian function, endometrial pinopodes and embryo implantation. METHODS: For pinopode analysis, 60 female mice were randomly divided into two groups: control (vehicle) and experimental [metoclopramide (MCP) 200 µg per day]. Injections were given subcutaneously for 50 days, and then, normally cycling females were housed with male mice for copulation during proestrus. The animals were killed on the fifth day following coitus when the antimesometrium portions of the uterine horns were removed for endometrial analysis. Blood was collected for prolactin (PRL) determination. In the second experiment, 60 female mice were used to evaluate the ovarian function by measuring estrogen and progesterone levels and counting luteal bodies and oocytes in the oviduct and uterus during estrus. RESULTS: The highest pregnancy rates and the largest population of pinopodes were both found in the vehicle group (P < 0.01). Estrogen and progesterone levels in MCP-treated mice were lower than those in control mice (P < 0.05). Also, the number of implantations was significantly lower in the MCP-treated group compared with the vehicle group after embryo transfer (P < 0.001). CONCLUSION: PRL seems to have suppressive effects on ovarian function and the number of pinopodes; conceivably, hyperprolactinaemia has a negative effect on mouse embryo implantation.

Key words: embryo implantation/hyperprolactinaemia/metoclopramide/mice/pinopodes

Introduction

Prolactin (PRL) is a pleiotropic hormone associated with reproduction, growth and development, water and electrolyte balance, metabolism, behaviour and immunoregulation (Bole-Feysot et al., 1998). The involvement of PRL in mammalian reproductive functions has been widely recognized. In addition to its very well-known role in milk production (Lewis et al., 1971), PRL may have an action on human endometrium, because it appears to interfere with macrophage activity and the release of immunological factors that might result in pregnancy disturbances (Healy, 1991). In mice, Bronson et al. (1996) demonstrated that PRL might act on the endometrium by modifying the immunologic activity or the regulation of gland-secreted immune factors. It could determine trophoblast proliferation and endometrial embryo invasion in rodents (Bronson et al., 1996). However, the influence of hyperprolactinaemia on endometrial embryo implantation has not been clarified yet.

The embryo implantation period, when the endometrium is maximally sensitive to blastocyst stimulus for decidualization, is usually referred to as the ‘implantation window’ and is well defined in mice (Singh et al., 1996). In addition, it is known that it is the antimesometrial segment of the endometrium that first acquires sensitivity to embryo implantation (Makker et al., 1994; Nikas et al., 1995). However, the reason the antimesometrial side is the one that preferentially attracts the blastocyst is still unknown, except for the fact that this side is more vascularized than the mesometrial side (Finn and Porter, 1975). The disturbance of the antimesometrial region can result in low numbers of embryo implantations (Martel et al., 1991). Data on hyperprolactinaemia directly or indirectly affecting that endometrial region are lacking.

The pinopodes are structures found in cells located in the antimesometrial area in mammals, and their presence may reflect shifts in the window of endometrial receptivity to the embryo (Nikas et al., 1999b). Furthermore, various authors view pinopodes as markers of endometrial sensitivity for embryo implantation in rats and in humans (Martel et al., 1991; Massai et al., 1993; Edwards, 1995; Sarantis et al., 1988; Yoshinaga, 1988). In fact, the presence of pinopodes is
strongly correlated with implantation following embryo transfer (Nikas, 1999a; Nikas et al., 1999b). Pinopodes seem to be directly involved in the adhesion of the blastocyst to the endometrial surface (Bentin-Ley et al., 1999). Therefore, they may be extremely useful for assessing receptivity on an individual basis to optimize embryo transfer (Nikas, 1999a; Nikas et al., 1999b). However, whether pinopodes are important in the implantation process is controversial because of the adherence of embryos to non-pinopode-forming cells and the fact that other factors, such as the leukaemia inhibitory factor (LIF), colony-stimulating factor-1 (CSF-1) and interleukin-1 (IL-1), may play important roles in the cascade of events leading to implantation (Lindhard et al., 2002).

In scanning electron micrographs, rat pinopodes appear as separate masses of cytoplasm with folds and flanges above the microvilli of endometrial cells (Enders and Nelson, 1973). Also, Singh et al. (1996) described pinopodes as cup-like bulbous cytoplasmic protrusions involved in the pinocytosis/endocytosis of fluid and macromolecules from the uterine lumen, facilitating its closure and the apposition of blastocysts onto the luminal epithelium. Furthermore, it has been proposed that those pinopodes constitute the first firm contact areas between embryonic and maternal cells (Casimiri and Psychoyos, 1981). This is based on the presence of crater-like imprints on the implanting blastocysts, indicating interdigitation of pinopodes and trophoblastic cells (Casimiri and Psychoyos, 1981). Pinopodes are very sensitive to ovarian hormones (Murphy, 2000). Moreover, the cellular projections that constitute the pinopodes are abundant in the uterus on the fifth day of rat gestation and to a lesser extent in the uterus of delayed implantation (Enders and Nelson, 1973).

The aim of this study was to evaluate the effects of metoclopramide (MCP)-induced hyperprolactinaemia on endometrial embryo implantation in mice and to analyse the antisomestrial area focusing on the role of pinopodes in pregnant mice.

Materials and methods

A total of 188 virgin female and 68 male EPM-M1 Swiss mice from the Center for the Development of Experimental Models for Medicine and Biology of the Federal University of São Paulo—Escola Paulista de Medicina (UNIFESP-EPM)—were maintained under controlled conditions of temperature (22 ± 1°C) with 12-h light/dark alternate periods (lights on at 7 a.m.) and free access to a pelleted diet (Labina-Purina) and water ad libitum (Table I).

First experiment

The animals were kept undisturbed in the new environment for 2 weeks. Thereafter, vaginal smears were taken daily to evaluate the estrous cycle. Sixty 100-day-old female animals with three regular cycles of 4–5 days were then randomly divided into two groups: (i) control, which daily received the drug vehicle (0.2 ml NaCl 0.9% in distilled water, n = 30) and (ii) experimental, which daily received 200 μg MCP dissolved in 0.2 ml saline solution (n = 30) (Singtripop et al., 1991). Injections were always given subcutaneously at 10 a.m., and the treatment lasted 50 days, during which time the estrous cycle was followed by vaginal smears. Next, the females were housed with male mice (one male for every two females). Copulation was confirmed by the presence of sperm in the vaginal mucus. The drug was administered until the fourth post-coital day. Females were killed by decapitation at 10 a.m. on the fifth post-coital day for blood collection. The median portions of the uterine horns were immediately removed and checked for the presence or absence of embryo implantation. The antisomestrial portions were dissected and washed in cold physiological saline solution and immediately fixed in fresh Karnowsky solution (Helder-Jose and Fremyuller, 1995). They were then post-fixed in 1% OsO₄ in the same buffer for 1 h, subsequently dehydrated in graded ethanol solutions and dried (Helder-Jose and Fremyuller, 1995). After coating with gold, the samples were examined with a JEOL JSM 5300 scanning electron microscope. The number of pinopodes was counted in a 100 μm² area of the scanning electron micrographic section of each mouse.

Second experiment

In a parallel experiment, the ovarian function of 60 additional female mice with regular estrous cycles (4–5 days) was evaluated by measuring the estrogen and progesterone levels in blood and counting the luteal bodies and oocytes from the oviduct and uterus during the estrous phase. For this purpose, the animals were randomly divided into two groups: (i) saline-treated controls and (ii) MCP-injected mice, as described above. After 50 days of treatment, 10 animals from each group were killed by decapitation during the estrous phase at 10 a.m. The blood from both groups was collected for estradiol (E₂) and progesterone determinations; the ovaries, the oviducts and the uteri of non-pregnant mice were removed for ovulation analysis. Forty females were housed with male mice (one male for every two females) for copulation, and the resulting pregnant mice (vehicle group, n = 18; MCP group, n = 16) were killed on the fifth post-coital day for blood collection. Evans Blue solution was then injected into the uterine arteries for checking the uterine implantation sides. Ovary fragments were processed by routine histological methods using haematoxylin–eosin stain. The luteal bodies were determined by counting them in 10 ovarian cross sections. The oviducts and the uteri were flushed with saline solution to remove the oocytes, which were then promptly counted with a Zeiss IM-35 microscope. The average number of luteal bodies and oocytes found on both sides for each animal was then calculated.

Blood samples were immediately centrifuged at 4°C (1500 g) for 10 min, and the supernatants were frozen at −20°C for subsequent analyses by radioimmunoassay (E₂ and progesterone) using a coated-tube, double-antibody technique (ICN Biomedicals, Costa Mera, CA, USA). The hormones were extracted from samples and incubated with ¹²⁵I-labelled E₂ or progesterone antibodies overnight at 4°C. The samples were assayed in duplicate and processed on the same day. In our conditions, the detection limits for E₂ and progesterone were approximately 0.1 pg/ml and 0.15 ng/ml, respectively. Cross-reactivities with other steroids for both assays were lower than 0.01%.

Third experiment

To evaluate whether the quality of the oocyte influenced the implantation, we performed an experiment using embryonic manipulation techniques with embryos of normal mice. Three- to five-week-old female mice were used for ovulation induction, mating and embryo

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Table I. Number of EPM-M1 Swiss mice used for each experiment

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Female</th>
<th>Male</th>
</tr>
</thead>
<tbody>
<tr>
<td>First (Tables II and III)</td>
<td>60</td>
<td>16</td>
</tr>
<tr>
<td>Second (Table IV)</td>
<td>60</td>
<td>17</td>
</tr>
<tr>
<td>Third (Total)</td>
<td>166</td>
<td>83</td>
</tr>
<tr>
<td>Total</td>
<td>288</td>
<td>116</td>
</tr>
</tbody>
</table>
donation, and male mice, about 8 weeks old, were used for copulation with the ovulation-induced females. Also, 6-week-old control female mice were treated for pseudo-pregnancy induction and used as recipients for the embryo transfer. Furthermore, 2-month-old male mice were vasectomized and used to induce the pseudo-pregnancy.

Ovulation induction of normal female mouse donors

The embryo transfer technique requires a large number of oocytes because at least 100 recently fertilized embryos are injected at each attempt. Intrauterine administration of gonadotrophins in prepubertal 3- to 5-week-old females induces ovulation induction with an average production of 30 oocytes per female. Initially, each female received 5 IU of pregnant mare’s serum gonadotrophin, which mimics FSH, and after 42–48 h 5 IU of HCG, which in turn mimics the LH peak. This hormone matures the follicles and induces the discharge of oocytes from the ovaries into the oviduct. On the same day as the HCG injection, each female was placed separately with a fertile male \( n = 40 \), and on the next day, the presence of a vaginal plug (mucus with spermatozoa) resulting from copulation is checked. Over 80% of the females \( n = 38 \) had a positive plug.

Collection of fertilized oocytes

The 32 females with a vaginal plug were killed through cervical displacement, and their abdominal cavities were opened to remove the oviducts, which were transferred to 35-mm plate with media. Generally, 15 embryos per ovary are produced, and at this phase, they are enveloped by a thick cellular mass called cumulus oophorus made up of granular cells and a matrix whose main component is hyaluronic acid. Under a stereomicroscope with 20× magnification, a small rupture was made on the oviduct wall with an ordinary needle, spontaneously exposing the cumulus oophorus because of the inner pressure of the oviduct, which was filled with liquid. The next step was to clean the embryos whose total mass was initially placed on a slide covered with a hyaluronidase enzyme culture that hydrolyzes the hyaluronic acid causing the matrix and the granular cells to expand or ‘soften’, making it easier to isolate the embryos. Then, with a pipette whose calibre was only slightly larger than the diameter of the embryos, the embryos are washed in culture medium and maintained in an oven with a 5% CO\(_2\) environment at 37°C until the time for transfer by injection.

Preparation of embryo recipients

The embryos were transferred to the oviducts of the pseudo-pregnant recipients, which were prepared by mating at least 6-week-old normally estrous females with vasectomized males. On the day before the transfer of the embryos, the vaginal plugs of 126 (60 and 66 vehicle and MCP-treated animals, respectively) females are checked and then the animals are placed together with vasectomized males, two females for each male in a box. Because of the estrous cycle, 28 (15 and 13 vehicle and MCP-treated animals, respectively) females are expected to have a positive plug after mating.

Vasectomy technique

Vasectomized males of any lineage are necessary to induce pseudo-pregnancy in embryo recipients. Vasectomy surgery is a sterilization method in which the deferent canals (the ducts connecting the testicles to the penis) are cut, blocking the passage of the sperm. Obstruction of the ducts may be achieved through cauterization with a pair of warm tweezers or by tying the duct at two close points and then cutting the duct half-way in-between. After 1 week the males are placed in boxes with several females until at least three positive plugs are obtained. The females which tested positive must be isolated and, if none turns out to be pregnant, the males may be used to induce embryo recipients. A stock of 20 vasectomized males can be kept until they are 1 year old, then new animals must be vasectomized.

Transfer of embryos to the oviduct

The embryonic age and the uterine conditions of the embryo recipient must be carefully synchronized. One-cell to morula (16 cells) embryos were transferred to the oviduct of pseudo-pregnant females 0.5 days after mating. The transfer was performed on the same day of the microinjection. Before transfer, the embryos were evaluated according to their number of cells and morphological quality: the cytoplasm must be clear and the pellucid zone, intact, and there must be no cytoplasmatic fragments. Just before surgery, two transfer pipettes, one for each oviduct, must be prepared to contain 10 embryos each, culture medium and air bubbles to indicate whether the embryos were deposited in the infundibulum. The transfer surgery begins with i.p. anaesthesia of the female followed by a central dorsal opening of the skin. The skin and the cut are laterally displaced until the ovary, which is usually covered with adipose material, was located. The muscle was cut as far as the peritoneal cavity, and the ovary, the oviduct and part of the uterus are carefully externalized and mobilized with a pair of tweezers. Under a stereomicroscope with 20× magnification, the burse (the membrane covering the ovary and the oviduct) is broken with two pairs of tweezers, and after that the infundibulum (the opening of the oviduct near the ovary) was located. The pipette was inserted at the initial portion of the oviduct, and the embryos are deposited slowly. The organs are then repositioned in the peritoneum, and at this point, it was not necessary to suture the muscle. The procedure was the same for the other oviduct. The skin was closed with a clip, and the female was kept on a warm plate at 37°C until her recovery from anaesthesia. The females were killed on the fifth post-coital day for blood collection. Evans Blue solution is then injected in the uterine arteries for checking the uterine implantation sides.

Statistical methods

Data are expressed as mean ± SD. All statistical tests were done using GraphPad Prism version 3.0 for Windows (GraphPad Software, San Diego, CA, USA). The unpaired Student’s \( t \)-test was used for comparing results of serum PRL, E\(_2\) and progesterone levels as well as the number of luteal bodies. Other results were analysed using Fisher’s exact test. Differences were considered significant at the \( P < 0.05 \) level.

Results

Vaginal smear analysis and PRL levels

To evaluate the estrous cycle before treatment, we collected vaginal smears daily. Most control or MCP-treated animals showed regular estrous cycles before and after treatment. In addition, both groups presented similar numbers of cycling and non-cycling mice during pretreatment and treatment periods (Table II). However, the MCP-treated group had longer estrous cycles (6–7 days) than the control group (4–5 days) during the treatment period.

A delay of the estrous cycle phase shift with a prolonged metoestrous plus diestrous phase was detected in the MCP-treated group (two more days in the metoestrous/diestrous phase).

Serum PRL levels of the MCP group were higher than those of the controls (551.5 ± 23.3 versus 130.4 ± 26.2 ng/ml, \( P < 0.001 \)).
Hyperprolactinaemia decreased pinopodes and pregnancy rates

Table II. Effect of metoclopramide on the estrous cycle (n = 30 per group)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cycling</td>
</tr>
<tr>
<td>Control (vehicle)</td>
<td>27</td>
</tr>
<tr>
<td>Metoclopramide</td>
<td>28</td>
</tr>
</tbody>
</table>

The definition of cycling mouse was based on the vaginal smear analysis. The animals were considered ‘cycling’ when they presented regular consecutive estrous cycles and had the following phases: pro-estrus, estrus, metaestrus and diestrus.

Embryo implantation

Table III summarizes the presence of implantation sites in cycling mice of both the vehicle and the MCP-treated groups. Pregnancy was much more frequent ($P < 0.001$, Fisher’s exact test) among the controls (78%) than the MCP-treated mice (21%).

Electron microscope scanning

Figures 1 and 2 show electron-scanning micrographs of the antimesometrial portion of the uterine lumina of non-pregnant mice. The uterine antimesometrial side of control animals had more epithelial microvilli with pinopodes ($0.13 \pm 0.02$ pinopodes/$\mu m^2$) than the MCP-treated mice ($0.06 \pm 0.01$ pinopodes/$\mu m^2$, $P < 0.01$, Figure 3). Also, the size of pinopodes in vehicle-treated animals was larger than those in MCP-treated ones (Figures 1 and 2). In both groups, we found a few ciliated cells intermingled with non-ciliated cells in some areas of the mouse endometrium.

The pregnant mice of the MCP-treated group had normal epithelia with microvilli, but the number of pinopodes ($0.09 \pm 0.02$ pinopodes/$\mu m^2$, Figure 4) was lower than that of the vehicle-treated group ($0.38 \pm 0.02$ pinopodes/$\mu m^2$, $P < 0.01$, Figures 3–5). In both groups, we found some ciliated cells intermingled with non-ciliated cells. In fact, these cells were clearly apparent on the apical antimesometrial side of the mouse uterine lumen on post-coitus day 5. Additionally, we found just a few non-ciliated cells of the MCP group on the antimesometrial side of the uterine lumen on the fifth post-coitus day.

Evaluation of luteal bodies, oocytes and ovarian hormones

Table IV summarizes the morphometric analysis of mouse luteal bodies, performed on ovarian cross sections, and the ovulated oocyte counts. No remarkable differences were noticed using light microscopy examination of the non-pregnant mouse ovaries. The circulating levels of E$_2$ and progesterone at the time of killing were significantly lower in the MCP-treated group compared with the vehicle group ($P < 0.05$, Table IV). But the hormone levels were similar in the pregnant mice of both groups on the fifth day following coitus (Table IV).

Evaluation of embryo implantation

Table V summarizes the presence of implantation sites in mice of both the vehicle and the MCP-treated groups after embryo transfer (of normal mice). Pregnancy was much more frequent ($P < 0.001$, Fisher’s exact test) among the controls (87%) than the MCP-treated mice (15%). Also, the number of implantations was significantly lower in the MCP-treated group compared with the vehicle group ($P < 0.001$).

Discussion

Actions related to the process of female reproduction represent the largest group of different functions that have been identified...
Figure 3. Number of pinopodes in non-pregnant and pregnant mice injected subcutaneously daily with 200 μg metoclopramide or vehicle for 50 days before mating.

* p < 0.01 compared to vehicle in both non-pregnant and pregnant mice

Figure 4. Scanning electron photomicrograph of the antimesometrial portion of the uterine lumen of a pregnant mouse in the vehicle group on day 5 following coitus, showing a large number of pinopodes (P) and microvilli (scale bar, 1 μm).

Figure 5. Scanning electron photomicrograph of the antimesometrial portion of the uterine lumen of a pregnant mouse in the metoclopramide group on day 5 following coitus, showing a low number of pinopodes (P) and microvilli (scale bar, 1 μm).

Table IV. Luteal body and ovarian hormone analyses

<table>
<thead>
<tr>
<th></th>
<th>Estrous phase</th>
<th>Day 5 post-coitus</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vehicle</td>
<td>Metoclopramide</td>
<td>Vehicle</td>
</tr>
<tr>
<td>Luteal bodies*</td>
<td>7.1 ± 1.3</td>
<td>6.7 ± 1.2</td>
<td>—</td>
</tr>
<tr>
<td>Ovulated oocytes b</td>
<td>6.4 ± 1.3</td>
<td>5.9 ± 1.9</td>
<td>—</td>
</tr>
<tr>
<td>Estradiol (pg/ml)</td>
<td>46.2 ± 2.2</td>
<td>39.4 ± 1.1*</td>
<td>83.6 ± 6.1</td>
</tr>
<tr>
<td>Progesterone (ng/ml)</td>
<td>2.8 ± 0.2</td>
<td>2.3 ± 0.1*</td>
<td>24.1 ± 0.9</td>
</tr>
</tbody>
</table>

aData represent mean ± SD of luteal bodies counted in ten ovarian sections.
bData represent mean ± SD of ovulated oocytes in the fallopian tubes.

*P < 0.05 compared with vehicle (unpaired t-test).

Table V. Presence of embryo implantation of normal mice after embryonic manipulation techniques and number of implantations in vehicle and metoclopramide groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Presence of embryo implantation [n (%)]</th>
<th>Number of implantation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Vehicle (n = 15)</td>
<td>13 (87)</td>
<td>2 (23)</td>
</tr>
<tr>
<td>Metoclopramide (n = 13)</td>
<td>2 (15)*</td>
<td>11 (85)</td>
</tr>
</tbody>
</table>

*P < 0.001, Fisher's exact test, comparing the MCP-treated group to the vehicle group.

**P < 0.001 compared with vehicle (unpaired t-test).

for PRL in rodents: (i) nurturing of young, (ii) development and secretory function of ovarian follicles and corpora lutea and (iii) promotion of blastocyst implantation (Richards, 1994; Binart et al., 2000). PRL is crucial for luteal function and maintenance of pregnancy (Telleria et al., 1997) and affects ovarian progesterone secretion as well as progesterone, receptor expression in uterine epithelium (Smith, 1980; Rothchild, 1981). PRL and its receptors are expressed in the uterus (Roby
et al., 1993; Reese et al., 2000) and the placenta, suggesting that this hormone may also have some potential for a paracrine/autocrine effect, like the stimulation of cell proliferation. Although hyperprolactinaemia can disturb female reproduction, its role in endometrial morphology changes is not clearly understood. In fact, our data show that MCP-induced hyperprolactinaemia negatively affects embryo implantation, partially by inhibiting pinopode formation, which may be an important structure allowing embryo attachment.

The first experiment was designed to study the effects of a pituitary-derivated hyperprolactinaemia on the number of pinopodes, because these structures might be responsible for embryo implantation. In fact, pinopodes are structures that anchor the embryo to maternal cells, and thus, some changes in the pinopodes may affect blastocyst implantation (Casimiri and Psychoyos, 1981; Nikas, 1999a; Nikas et al., 1999b). However, other authors have shown embryo adherence to non-pinopode-forming cells. The embryo binds to the apical sides of two adjacent endometrial cells, which then separate, opening up like a zipper and giving access to the trophoblastic protrusions along the lateral plasma membranes. It appears that the formation of junctions at the lateral compartments of endometrial epithelial cells provides sufficient stability for the trophoblast to induce cell separation (Bentin-Ley et al., 2000). The ovarian steroids may affect the presence of ciliated/non-ciliated cells in the endometrium. Bharatiya and Bajpai (1995) demonstrated that oestradiol during the oestrogenic phase induces an increase in the number of ciliated cells; by contrast, progesterone, in addition to inducing secretory activity in non-ciliated cells, had a negative effect on oestradiol-dependant morphological characteristics of ciliated cells, causing a reduction in cell number and deciliation.

One possible explanation for PRL effects on the endometrium is a direct effect through the PRL receptor, and another is an indirect effect through a decrease in the ovarian hormonal production (Arredondo and Noble, 2006). In fact, our data show a reduction in the E2 and progesterone levels during the estrous phase in the MCP-induced hyperprolactinaemia in mice. This effect may underlie the reduction in the number of embryo implantations. However, a study showed that high serum PRL levels might indicate interference in follicular and oocyte development, leading to oocytes of inferior quality (Reinthaller et al., 1987). In contrast, a transient hyperprolactinaemia is positively associated with ICSI outcome, in particular with oocyte quality and fertilization rate (Doldi et al., 2000). Regardless of the possibility that hyperprolactinaemia affects oocyte physiology, our data indicate that endometrial changes may interfere with transferred embryo implantation in MCP-treated mice and show that the number of ovulated oocytes and luteal bodies were similar in MCP-treated and vehicle groups.

Previous studies showed that MCP-induced hyperprolactinaemia affects female mouse physiology through changes in the estrous cycle, leading to a prolongation of the diestrous phase or a disruption of the cycle (Singtripop et al., 1991; Rossi et al., 2002). Hyperprolactinaemia may also induce a decrease in estrogen production and LH release, resulting in an anovulatory state and non-cycling mice (Dorrington and Gore-Langton, 1981; Kalion et al., 1985). In fact, our data mainly show a delay of the estrous cycle phase shift with a prolonged metoestrous plus diestrous phase, an event which may be related to the decrease in estrogen level (Dorrington and Gore-Langton, 1981).

In rodents, the process of implantation involves complex interactions and requires a precise coordination between the establishment of uterine receptivity and blastocyst activation (Chakraborty et al., 1996). This process is primarily dependent on the concerted effects of steroids (Psychoyos, 1973). At the beginning of pregnancy, pre-ovulatory ovarian E2 directs epithelial cell proliferation (Given and Enders, 1980). Consequently, a decrease in estrogen level may influence uterine receptivity.

Progesterone is needed for the development of pinopodes in rodent (Singtripop et al., 1991; Singh et al., 1996) and human (Stavreus-Evers et al., 2001) endometria. Thus, embryo implantation might be related to the number of pinopodes and the circulating progesterone levels (Reese et al., 2000). This fact may explain the reduction in the number of blastocyst implantations of the MCP-treated group. However, other data suggest that PRL acts as an immunocrine hormone in the endometrium (Sabharwal et al., 1992). The LIF, CSF-1 and IL-1 may be involved in the disturbance of events leading to implantation (Lindhard et al., 2002).

In conclusion, we postulate that MCP-induced hyperprolactinaemia negatively affects ovarian function, endometrial morphology and embryo implantation in mice.

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References


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