Effect of GnRH on guinea pig endometrium at preimplantation stage

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Endometrium of GnRH treated group resembled with pregnant group and endometrial thickness in these groups significantly increased in comparison with non-pregnant group. In GnRH treated animals, most of histomorphological changes in epithelial cells, glands and stroma of uterus was similar to pregnant group. The results revealed that mammalian form of GnRH exerted endometrial change in guinea pig almost similar to those occur in normal pregnant animals and its administration prior to implantation may improve pregnancy rate following embryo transfer.

Keywords: GnRH, Guinea pig endometrium, Preimplantation stage

The hypothalamic decapeptide gonadotropin-releasing hormone (GnRH) is the key neuromodulator of mammalian reproduction. This hormone is released from the hypothalamus via the hypophysial portal system and binds to specific receptors on the anterior pituitary, resulting in the stimulation of biosynthesis and release of follicle stimulating hormone (FSH) and luteinizing hormone (LH). A variety of tissues in several mammalian species express an extra hypothalamic GnRH that is immunologically, biologically and chemically identical to the hypothalamic hormone. Moreover, the presence of low affinity/ high capacity binding sites for GnRH has been demonstrated in several extra pituitary organs, e.g. the placenta, endometrium, myometrium, breast, prostate, ovary and testis. GnRH and its receptor have been detected in pre- and peri-implantation embryos, the fallopian tubes, and uterine endometrium in different species. Their potential role in embryo development, endometrial preparation, and the implantation process has also been previously suggested in both mouse and human. Strong evidence exists for GnRH analogues affecting embryonic development.

Preimplantation embryonic development and implantation is a complex series of steps that under normal circumstances begins even before the blastocyst reaches the uterine cavity and attaches to the endometrium. In order to complete this enigmatic process, there is an embryonic-maternal dialogue, in which the embryo and the endometrium induce changes in each other to promote embryonic development and endometrial receptivity. For successful implantation, embryo-endometrial interaction must be initiated when the embryo and endometrium have reached precise stages of development and hormone dependent changes resulting in the development of a short lived receptive endometrium must have occurred. In presence of GnRH agonist, the proportion of mouse embryos reaching hatching blastocyst stages in vitro is increased. Moreover, GnRH antagonist had a detrimental effect on preimplantation embryonic development, which was totally reversed by the addition of GnRH agonist, suggesting a specific action on embryo development, rather than a non-specific or toxic effect. Several reports concerning inadvertent administration of GnRH agonist during early pregnancy have been described, and a possible positive role for GnRH in human early implantation has been suggested.

Low implantation rates following in vitro fertilization and embryo transfer is a concern. Whether this low rate is a consequence of an inherently low implantation rate in human or to an
altered physiological state is presently unknown. Based on experimental animals, there are reasons for suspecting that the low implantation rate may be consequence of an altered physiological state. Among non-primates, guinea pig is the best available laboratory animal as a model for study of human implantation. Hence this study has been performed to compare histological changes of endometrium in non pregnant, pregnant and GnRH treated animals at preimplantation stage.

Materials and Methods

Animals—Fifteen sexually mature female guinea pig (Cavia porcellus) weighing 500-600 g and 4 months of age were obtained from animal house of Shiraz Medical University. Animals were randomly classified in three groups of non-pregnant (control negative), pregnant (control positive) and GnRH treated, each, caged individually under controlled light (14:10 hr light/dark) and temperature (22°C-25°C) and were given standard pelleted food and water ad libitum. All studies were performed in accordance with National Institutes of Health Guide for the care and use of Laboratory Animals.

Experiment design—Estrous cycle of animals was checked by vaginal smear. Animals at estrus stage were selected for experiment and grouped as pregnant (control positive), non-pregnant (control negative), and GnRH treated. Animals of pregnant group were caged with male for mating following detection of estrus while, non pregnant and GnRH treated groups were not allowed to mate. GnRH treated group received GnRH (20 μg/kg body weigh) on second day of estrus cycle. All animals were anaesthetized on day 7th of estrous cycle (expected day of implantation), their uteri were isolated and two pieces from each horn dissected and transferred to 10% formalin, following washing with normal saline. Tissue processing was carried out by autotechnicon. Section 5-6 μm thick mounted on slide and stained with Hematoxylin/Eosin or Green Masson’s Trichrome. For histomorphological and histomorphometric study the sections were observed under light microscope. Among endometrial layers, changes of epithelial cells, growth of endometrial gland, presence of inflammatory cells and angiogenesis were evaluated. Minimum and maximum thickness of endometrium was also measured in all groups.

Statistical analysis—Normal distribution of data were tested by kolmogorov smirnov (K-S) and the data were compared with one-way analysis of variance (ANOVA) and Post Hoc test.

Results

In the uterine cross section of control negative group few crypts were seen, epithelial layer of endometrium consist of low columnar cells, nuclei positioned on basal border of cells, with no secretion in their cytoplasm. In endometrial stroma most of glands are non-functional without secretion and epithelium of these glands is surrounded by low columnar cells (Fig. 1). In pregnant group epithelial layer of endometrium changed from low columnar to high columnar and hypertrophied cells (Fig. 2), the uterine glands are active, tortured, filled with secretion and surrounded with high columnar cells, expansion of blood vessels and angiogenesis is another obvious change of pregnant uterus (Fig. 3). In GnRH treated animals most of histomorphological changes in epithelial cells, glands and stroma of uterus was similar to pregnant group (Figs 4 and 5). The thickness of endometrium in GnRH treated and pregnant group was found to be significantly (P< 0.05) increased as compared to non pregnant group, while the difference between GnRH treated and pregnant group was non-significant (Fig. 6).

Discussion

Three types of GnRH have been identified; mammalian (mGnRH), Chicken (cGnRH) and guinea pig (gpGnRH). In most vertebrates multiple GnRH variants expressed in a single species. In the present study mammalian GnRH which is used in cattle was administrated in guinea pig and could induce endometrial changes similar to those which occur in pregnant animals. The biological activity of synthetic gpGnRH and mGnRH on LH and FSH secretion was studied in two in vitro rat cell culture models and showed similarity in term of potency, but with different sensitivity. Guinea pig is potentially useful alternative laboratory animal because several aspects of its reproductive function are more similar to those of primate than to those of mice or rats.

GnRH receptors are present in uterus and recent reports suggest positive effect of GnRH on embryo development. GnRH is thought to be an autocrine and paracrine regulator in the reproductive tissues. The logic objective of this study was to evaluate and compare structural changes of endometrium during early pregnancy, at preimplantation stage, and
Figs 1-5—(1) Endometrium of non-pregnant guinea pig (Masson's Trichrome X520); (2) Endometrium of pregnant guinea pig (Masson's Trichrome X520); (3) Endometrial gland of pregnant guinea pig (H&E X520); (4) Endometrium of GnRH treated guinea pig (Masson's Trichrome X520); and (5) Endometrial gland of GnRH treated guinea pig (H&E X520)
following administration of GnRH. The uterine sections were collected and prepared when the animals were at diestrus stage, the epithelial cells of endometrium of non-pregnant group did not have secretion and the endometrial gland showed inactive appearance. This morphological feature is quiet rational and could be due to alteration of progesterone and estrogen during diestrus23. This finding supports the report by Alkhalaf et al24.

In pregnant group secretory activity of cells and accumulation of secretions in cytoplasm pushed the nucleus to apical border of cells. Increased number of glands and torturous appearance of glands in pregnant group is mostly due to steroidal hormones increment required for preparation of endometrium for implantation24,25.

In GnRH treated group, morphology of endometrial and its thickness changed and its general appearance transformed as pregnant uterus, which makes endometrium suitable for implantation. Implantation is a complex series of steps that under normal circumstances begins even before the blastocyst reaches the uterine cavity and attaches to the endometrium. In order to complete this enigmatic process, there is an embryonic–maternal dialogue, in which the embryo and the endometrium induce changes in each other to promote embryonic development and endometrial receptivity12. The results of the present study support the report of Garcia et al26 and Ubaldi et al27 about positive effect of GnRH on human endometrium. Positive effect of GnRH on endometrium may be due to its direct effect on uterine receptors22 or its action on pituitary and enhancing LH or FSH secretion, which consequently will affect ovarian and endometrial secretions. Both mGnRH and gpGnRH are present in domestic guinea pig18 and pituitary cells of domestic guinea pig have higher sensitivity to mGnRH than gpGnRH both in vitro and in vivo5. Moreover, it has been shown that the use of high doses of GnRH antagonist is associated with low implants rates in IVF indicating positive effect of GnRH on endometrium28.

In conclusion, mammalian form of GnRH could exert endometrial changes in guinea pig almost similar to those occur in normal pregnant animals and its administration prior to implantation may improve pregnancy rate following embryo transfer.

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