Iranian Journal of Reproductive Medicine Vol.4. No.1 pp:7-11, May 2006

The impact of ovarian stimulation on mouse endometrium: a morphometrical study

Mojdeh Salehnia, Ph.D., Mitra Arianmanesh, M.Sc., Mandana Beigi, M.Sc.
Department of Anatomy, Faculty of Medical Sciences, Tarbiat Modarres University, Tehran, Iran.
Received: 2, October, 2005; accepted: 20, April, 2006

Abstract

Background: The preparation of endometrium for embryo reception is dependent on the ovarian hormones, which are affected by ovarian hyperstimulation procedure.

Objective: The aim of this study was to evaluate the changes in morphometrical indices of endometrium by the daily injection of progesterone after mouse ovarian induction.

Materials and Methods: Adult virgin female mice were selected and divided into control and experimental groups. Experimental groups were superovulated using human menopasual gonadotropic hormone (HMG), and human chorionic gonadotropic hormone (HCG), then they, were subdivided into two groups, which one group was also injected daily by progesterone. All control and hyperstimulated groups were rendered pseudopregnant by cervical stimulation. Three and four days after the HCG injection, the samples of uterine horns were aparted and processed for light microscopic studies.

Results: Our results showed that in the progesterone-injected group, the height of surface and glandular epithelium was decreased on day three (17.6±3.55, 10.02±2.6) and day four (16.9±4.24, 1.6±0.84) respectively, and it had low columnar morphology in comparison with the hyperstimulated and control groups. Also the intercellular spaces of stroma in progesterone-injected group were narrower than these in the other groups.

Conclusion: Ovarian hyperstimulation followed by progesterone injection alter the morphometrical indices of surface and glandular epithelium of endometrium, which could affect on its receptivity.

Key words: *Endometrium, Morphometry, Ovarian stimulation, Progesterone*

Introduction

Implantation is a complex sequence of processes between the embryo and endometrium. The surface of embryo and endometrium undergoes a series of changes within a short time, which is considered as "implantation window". During this time, the endometrium has high efficiency for receiving the embryo (1,2). These changes have been observed on the morphology, ultrastructure and molecular levels of endometrium (3,4). At the time of embryo adhesion, the microvilli are replaced with another fungi form cytoplasmic projections named as pinopodes. These swelling projections have been appeared for a short time (24-48 hours) at the endometrium surface and assumed as uterine receptivity markers in some mammals (3-5). The effect of progesterone on endometrium receptivity is clear. This hormone is needed to create typical luteal changes and the secretary stage of the endometrium during the decidual reaction (6,7). The preparation of endometrium for embryo reception is dependent on the ovarian hormones which are affected by ovarian hyperstimulation procedure (8).

There are some regimes for ovulation induction and hormones replacement therapy such as progesterone administration after human chorionic gonadotropic hormones (HCG) injection for the maintenance of corpus luteum and preparation of endometrium for embryo transfer. After the administration of exogenous gonadotropin hormone to obtain a large numbers of oocytes, the secretion of oestrogen and progesterone increases (9). Investigations on human and experimental animals showed that after hyperstimulation, the implantation rates declined in comparison with the normal groups (8-11). Fossum *et al* (9) reported a significant decrease in the implantation rates after embryo transfer to ovarian stimulated mice using Pregnant Mare Stimulating Gonadotropin (PMSG) and HCG and suggested that this failure was caused by changes in uterine receptivity (9). In Karmer *et al* (12) study a

---

Correspondence Author:
Dr. Mojdeh Salehnia, Department of Anatomy, Faculty of Medical Sciences, Tarbiat Modarres University, P.O.Box: 14115-111, Tehran, Iran.
E-mail: mogdeh@dr.com

high luteal phase oestradiol/progesterone ratio has been associated with implantation failure in mice. Basir *et al* (10) concluded that excessive high concentration of oestradiol leads to suboptimal endometrial environment for implantation and this may explain the findings regarding the decreased implantation and pregnancy rates in IVF.

Since the surface and glandular epithelial thickness depends on the ovarian hormones, it is suggested that some morphometric indices of endometrium should be changed after ovarian induction regimes. Previous researches showed a delay in maturation of endometrium epithelium and stroma after ovarian stimulation in human and animals (10, 13-15).

The main question is that if the high level of oestrogen and progesterone concentrations after ovarian hyperstimulation and progesterone injection (as replacement therapy) does influence the structure of endometrium at the peri-implantation period? The purpose of this study was to investigate the alterations in some morphological indices of mouse endometrium after hyperstimulation using HMG and HCG injections followed by the daily injections of progesterone at the implantation time.

Materials and Methods

Animals

Female virgin NMRI mice, aged 6-10 weeks, were cared for and used according to the guide for the care and use of laboratory animals and housted under 12h light: 12h dark condition. They were randomly divided into three groups:

Group A: control group, which were rendered pseudopregnant by cervical stimulation (16).

Group B: hyperstimulated mice, which were superovulated using an intraperitoneal injection of 10 i.u. HMG (Sereno) followed by another injection of 10 i.u. HCG (Organon) 48 hours later. On the evening of the second injection, the mice were rendered pseudopregnant the same as the control group.

Group C: hyperstimulated mice with progesterone administration, which superovulation the same as group B, then daily subcutaneous injections of progestrone (1 mg/mouse) were performed (17) and the mice were rendered pseudopregnat the same as the other groups.

Tissue preparation

Thirty mice from each group were sacrificed by cervical dislocation on 3 (pre implantation time in mice) and 4 (implantation time in mice) days after HCG injection. The samples were obtained from the middle 1/3 part of their uterine horns immediately and processed for the following studies.

Morphometrical study

Five tissues from each group, on third and forth day were fixed in formaldehyde, embedded in paraffin wax, sectioned at 6 micrometer and stained using hematoxyline and eosin technique.

After preparation of the sections, 3 slides were chosen randomly from each sample and at least four fields of view were measured from each slide. The following endometrial parametres were measured in each field of view: (I) the surface epithelial cell thickness (µm) from the luminal border to its basement membrane; (II) the glandular epithelial cell thickness (µm) from the luminal border to its basement membrane; (III) the endometrial thickness (µm) from the luminal border of the epithelium to the upper layer of the myometrium and (IV) the gland diameter (µm) (18) . The measurments on each slide were made using the 40 times objective of a Zeiss microscope with a calibrated eye piece.

Statistical analysis

Data were collected from each group and the mean±SD was calculated. Groups were compared using student t-test. Data were analysed using SPSS softwares.

Results

At the light microscopic levels, the morphology of the surface epithelium in the control, hyperstimulated and hyperstimulated-progesterone injected groups were simple columnar, pseudostratified columnar and simple low columnar, respectively.

The morphometric data on three and four days after HCG injection (table I and II) showed that the surface epithelial cell thickness on the third and fourth days of HCG injection was decreased in the hyperstimulated groups (18.58± 3.5 µm, 23.67± 4.18 µm) compared with the non-stimulated group (23.57± 4.31 µm, 38.40± 2.88 µm) ($p= 0.0001$). The hyperstimulated-progesterone injected group had lower epithelial cell thickness on days three (17.16± 3.55 µm) or four (16.92± 4.24 µm) after pseudopregnancy in comparison with the control and hyperstimulated groups ($p= 0.0001$). These data demonstrated that the ovarian induction, which was followed by progesterone administration, influenced the endometrial thickness. Similarly, there were statistically significant differences between the glandular cell thickness in hyperstimulated (11.52± 2.65 µm, 9.4± 1.66 µm), hyperstimulated-progesterone injected (10.02± 2.6 µm, 12.06± 2.84

µm) and the control groups (14.58± 2.77 µm, 23.35± 4.3 µm) respectively (p= 0.0001).

The mean diameter of glands, three days after HCG injection in the control, hyperstimulated and hyperstimulated-progesterone injected groups were 39.48± 7.85 µm, 33.88± 7.29 µm and 36.26± 7.57 µm, respectively, which showed no significant differences among these groups. But on the fourth day of HCG injection, the mean diameter of glands was greater in the control group (52.20± 9.11 µm) compared to the hyperstimulated (33.33± 8.14 µm) and hyperstimulated-progesterone injected groups (39± 8.7 µm) (p=0.0001).

The endometrial thickness on the third day of pseudopregnancy in the control, hyperstimulated and hyperstimulated-progesterone injected groups was 234.96± 49.95 µm, 238.56± 38.62 µm and 209.27± 54.33 µm respectively and there were no significant differences among these groups. Whereas, on the fourth day of pesudopregnancy, there was significant difference between the control (276.48± 41.21 µm) and the hyperstimulated-progesterone injected group (230.08± 65.52 µm; p=0.001) and also there was significant difference between the latter group and the hyperstimulated group (265.38± 59.98 µm; p= 0.013).

The stroma of both hyperstimulated and progesterone injected groups were compact and their intercellular spaces were narrower than the control group (fig1).

Table I. Morphometric assessment of the stimulated and control mouse endometrium three days after HCG injection and pseudopregnancy

Endometrial Morphometric Parameters		Control	Hyperstimulated	Hyperstimulated-progesterone injected
Surface epithelial cell height (µm)	Mean ± SD	23.57 ± 4.31	18.58 ± 3.5[a]	17.16 ± 3.55 [b]
	Range	(14-34)	(12-24)	(12-24)
Glandular epithelial cell height (µm)	Mean ± SD	14.585 ± 2.77	11.52 ± 2.65 [a]	10.02 ± 2.6 [b]
	Range	(9.6-21.6)	(7.2-19.2)	(7.2-16.8)
Gland diameter (µm)	Mean ± SD	39.48 ± 7.85	33.88 ± 7.29	36.26 ± 7.57
	Range	(43.27-52)	(24-48)	(22-53)
Endometrial thickness (µm)	Mean ± SD	234.96 ± 49.95	238.56 ± 38.62	209.27 ± 54.33
	Range	(144-360)	(168-312)	(103-319)

a: Significant differences between the control and hyperstimulated groups (p< 0.05).
b: Significant differences between the control and hyperstimulated-progesterone injected groups (p< 0.05).

Table II. Morphometric assessment of the stimulated and the control mouse endometrium four days after HCG injection and pesudopregnancy

Endometrial Morphometric Parameters		Control	Hyperstimulated	Hyperstimulated-progesterone injected
Surface epithelial cell height (µm)	Mean ± SD	38.40 ± 2.88	23.67 ± 4.18 [a,c]	16.92 ± 4.24 [b,c]
	Range	(31-43)	(17-36)	(10-26)
Glandular epithelial cell height (µm)	Mean ± SD	23.35 ± 4.3	9.4 ± 1.66 [a,c]	12.06 ± 2.84 [b,c]
	Range	(16.8-36)	(7.2-12)	(7.2-19.2)
Gland diameter (µm)	Mean ± SD	52.20 ± 9.11	33.33 ± 8.14 [a]	39 ± 8.7 [b]
	Range	(46-72)	(24-48)	(19-55)
Endometrial thickness (µm)	Mean ± SD	276.48 ± 41.21	265.38 ± 59.98	230.08 ± 65.22 [b,c]
	Range	(209-367)	(144-360)	(164.8-360)

a: Significant differences between the control and hyperstimulated groups (p< 0.05).
b: Significant differences between the control and hyperstimulated-progesterone injected groups (p< 0.05).
c: Significant differences between the hyperstimulated group and hyperstimulated-progesterone injected group (p< 0.05).

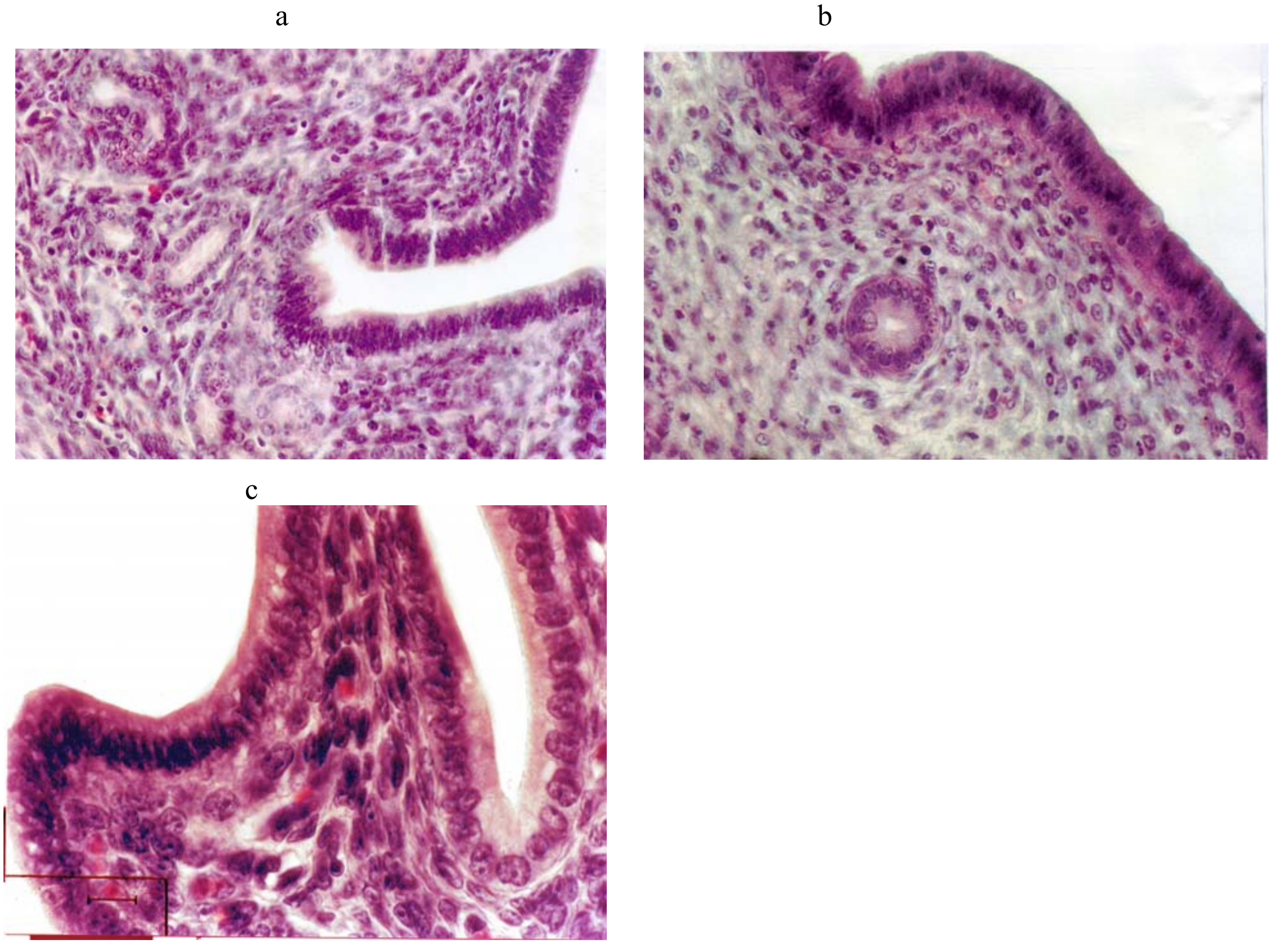

Figure1. The mouse endometrium on the 4th day of pregnancy in (a) control, (b) hyperstimulated, (c) hyperstimulated-progesterone injected group. Magnification is 1000.

Discussion

Our observation showed that in the hyperstimulated-progesterone injected group, the height of epithelium was decreased in comparison with the control and hyperstimulated groups. These changes may be due to the alteration in the ratio of progesterone to oestrogen, which caused a reduction in the cytoplasm and / or changes in the volume of the nucleus. Risek *et al* (19) showed that progesterone injection to immature rats decreased the height of endomerial epithelium. The elevated progesterone level may cause the decline in endometrial receptivity, which was previously showed after ovarian hyperstimulation (12,19).

Dursum *et al* (20) showed exogenous administration of gonadotropins significantly affects the morphology of the endometrium and the mitotic index in the implantation period of the embryo.

These morphological effects became more pronounced when the administrated dose of exogenous gonadotropins was increased.

In addition, our results showed that in both hyperstimulated groups the stroma is compact therefore, the decidualizations were defective in hyperstimulated groups. In agreement with our results, Kramer (21) showed that in ovarian hyperstimulated rats no decidualization reaction was seen. He concluded that it was due to the decrease in vascular permeability (21). Also Stein and Kramer (18) showed stromal cells in hyperstimulated rats ovary failed to undergo decidualization. McRae and Heap (22) reported that in ovariectomised rats under progesterone treatment, the number of permeable vessels was decreased, whereas after the treatment of these animals with oestrogen, the permeability of vessels was increased. They concluded that progesterone controlls the permeability of these vessels. Kramer (21) showed that the ratio of progesterone to estrogen before implantation in the hyperstimulated groups was low which was probably due to a decrease in the permeability of the vessels.

In contrast to our results, Kolb *et al* (23) speculated that high levels of progesterone in the early luteal phase of cycles, undergoing controlled hyperstimulation, caused premature endometrial luteinization and a premature appearance of the implantation window. In addition, our group reported previously (24) that the progesterone injection following ovarian induction could cause premature expression of endometrium pinopodes before implantation time. Thus, ovarian hyperstimulation with or without progesterone injection alter the thickness of the surface and glandular epithelium of endometrium, which could affect the endometrial receptivity.

References

1. Leesey BA. The role of the endometrium during embryo implantation. *Hum Reprod* 2001; 15, 36-50.
2. Nikas G. Endometrial receptivity: changes in cell- surface morphology. *Semin Repord Med* 2000; 18, 229-235.
3. Creus M, Ordi J, Fabregues F, Casamitjana R, Carmona F, Cardesa A, *et al*. The effect of different hormone therapies on integrin expression and pinopode formation in the human endometrium: a controlled study. *Hum Reprod* 2003; 18, 683-693.
4. Nikas G, Makrigiannakis A. Endometrial pinopodes and uterine receptivity. *Ann N K Acad Sci* 2003; 997, 120-123.
5. Murphy CR. The plasma membrane transformation: a key concept in uterine receptivity. *Rep Med Rev* 2001; 9, 197-208.
6. Hewitt SC, Korach KS. Progesterone action and responses in the αERKO mouse. *Steroid* 2000; 65, 551-557.
7. Sengupta J, Ghosh D. Role of peri-implasntation stage endometrium-embryo interaction in the primate. *Steroid* 2000; 45, 753-762.
8. Bourgain C, Devroey P. The endometrium in stimulated cycles for IVF. *Hum Reprod Update* 2003; 9, 515-522.
9. Fossum GT, Davidson A, Paulson RJ. Ovarian hyperstimulation inhibits embryo implantation in the mouse. *In Vitro Fert Embryo Transfer* 1989; 6, 7-10.
10. Basir GH, Wai-sum O, Hung Yu Ng E, Chung Ho P. Morphometric analysis of prei-implantation endometrium in patients having excessively high oestradiol concentration after ovarian stimulation. *Hum Rreprod* 2002; 16, 435-440.
11. Ertzeid G, Storeng R. The impact of ovarion stimulation on implantation and fetal development in mice. *Hum Reprod* 2001; 16, 221-225.
12. Kramer B, Stein BA, Van der Walt LA. Exogenous gonadotropins-serum oestrogen and progesterone and the effect on endometrial morphology in the rat. *J Anat* 1990; 173, 177-189.
13. Biegy M, Salehnia M, Tiraihi T. Delayed decidulaization and ultrastructural changes of mouse endometrium after mouse ovarian hyperstimulation at the implantation time. *Middle East Fertil Soc J* 2003; 8, 229-234.
14. Pellicer A, Valbuena D, Cano F, Remohi J, Simon C. Lower implantation rates in high responders evidence for an altered endocrine milieu during the implantation period. *Fertil Steril* 1996; 65, 1190-1195.
15. Thomas K, Thomson AJ, Sephton V, Cowan C, Wood S, Vince G, *et al*. The effect of gonadotrophic stimulation on integrin expression in the endometrium. *Hum Reprod* 2002; 17, 63-68.
16. Murdoch RN, Kay DH, Cross M. Activity and subcellular distribution of mouse uterine alkaline phosphatase during pregnancy and pseudopregnancy. *J Reprod Fertil* 1978; 54, 293-300.
17. Miller BG, Delayed interactions between progesterone a low doses of 17 β-estradiol in the mouse uterus. *Endocrinol* 1979; 104, 26-33.
18. Stein B, Kramer B. The effectof exogenous gonadotropichormones on the endometrium of the rat. *H Anat* 1989; 164: 123-140.
19. Risek B, Klier F G, Phillips A, Hahn D W, Gilula N B. Gap junction regulation in the uterus and ovaries of immature rats by estrogen and progesterone. *J Cell Sci* 1995; 108, 1017-1032.
20. Dursun A, Sendag F, Terek M C, Yilmaz H, Oztekin K, Baka M, Tanyalcin T. Morphometric changes in the endometrium and serum leptin levels during the implantation period of the embryo in the rat in response to exogenous ovarian stimulation. *Fertil Steril* 2004; 82 1121-1126.
21. Kramer B. Changes in vascular permeability and decidoma formation during the peri-implantation period of the rat in responsee to exogenous gonadotropins. *Anat Record* 1997; 247, 20-24.
22. McRae AC, Heap PB. Uterine vascular permeability, blood flow and extracellular fluid space during implantation in rates. *J Reprod Fertil* 1988; 82, 617-625.
23. Kolb BA, Najmabadi S, Paulson RJ. Ultrastructural characteristics of luteal phase endometrium in patients undergoing controlled ovarian hyperstimulation. *Fertil Steril* 1997; 67, 625-630.
24. Emadi M, Salehnia M. The morphological expression of endometrial pinopodes during implantation in mice after ovarian stimulation and progesterone injection. *Yakhteh Med J* 2004; 5, 140-145.