

## Review Article

# Experimental Endometriosis: Review of the Literature Through a Century and the Iranian Experience

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Although endometriosis is not a newly discovered disease, it has still remained enigmatic. Understanding the pathophysiology of this disease is still one of the challenges of gynecology and reproductive medicine. Therefore, the subject of endometriosis treatment faces many questions to be answered. Even though this is not considered to be a rare disease, testing novel treatments on subjects is definitely contrary to medical ethics. This is where importance of animal studies is emphasized. History of animal studies on endometriosis dates back to the time when the young John Sampson presented his theory of retrograde flow of menstruation to the gynecologic society of his time to explain the mechanism of endometriosis. However, most of the medical society of that time favored metaplasia as the main mechanism over the new theory. In order to prove Sampson's theory, animal models were used to induce endometriosis, and that was the first study of experimental endometriosis. Nowadays, although no one uses animals to evaluate Sampson theory of endometriosis, however, experimental endometriosis is widely used to study many different aspects of the disease from pathogenesis to possible options for treatments. Also, since then, various animals and different techniques have been proposed and so there is a huge body of literature on experimental endometriosis. Contrary to many countries, Iranian medical societies have neglected animal models for endometriosis until recently. This review article aims to go through the prominent articles on the subject and introduce different animals and methods to its readers and have a special look at Iranian literature on experimental endometriosis.

**Keywords:** Animal experimentation, Animal model, Endometriosis, Rats, Review**Cite this article as:** Alborzi S, Azimirad A, Azimirad M. Experimental endometriosis: review of the literature through a century and the Iranian experience. Arch Iran Med. 2018;21(11):536-543.

Received: March 7, 2018, Accepted: October 7, 2018, ePublished: November 1, 2018

**Introduction**

Endometriosis was first named by Von Recklinghausen in the year 1885.<sup>1</sup> This nomenclature effectively describes the basis of the disease; leaving nothing more to be said. However, "what really happens in details?" is the challenging question that faces the molecular medicine era scientists.

Until now, some vague explanations have been made about the pathogenesis of endometriosis, which none seems to satisfy the experts' attitudes for all the cases. The etiology of endometriosis has not been clear enough.<sup>2,3</sup> By comparing different reports, the molecular aspects of endometriosis look more like chaos. Making an accurate diagnosis needs aggressive approaches and there are controversial disputes about its treatment. Various protocols for treatment by various authorities and associations wax and wane from time to time, while no single consensus for treatment has been approved.<sup>3,4</sup>

This plurality in interpretation of endometriosis brightly reflects the lack of scientific body of evidence on the subject of endometriosis. On the other hand, endometriosis is considered a very common disease in gynecology which affects female wellbeing, quality of life, sexual life and

reproduction.<sup>5</sup>

In order to be more specialized on endometriosis, we need more endometriosis cases. On one side, comprehensive study of its physiopathology needs observation of cases from the onset of pathogenesis of the disease; of note, most cases of endometriosis cannot be diagnosed from the early onset. On the other hand, due to ethical issues about the use of human subjects in medical research, utilization of new pharmacologic and non-pharmacologic therapies and interventions without adequate, definite and secure results of *in vivo* and *in vitro* studies seems unacceptable.

These are simply the reasons behind a movement in laboratories which concluded in a field of science concerning about the development of different models of endometriosis by various techniques. In fact, talking about laboratory endometriosis even dates back to the time the disease was almost newly described.<sup>6</sup> Since then, this science has progressed into different branches. They include different *ex vivo*, *in vivo* and *in vitro* models, each employing different techniques.

Here, firstly, experimental endometriosis from the very first day of its development and its trials and errors in

various species to the establishment of structured methods are presented. Then, a detailed discussion of common animals as *in vivo* models is made; a practical manual for mimicking endometriosis in laboratory, focused on successful techniques for induction of endometriosis are presented. Scarce number of research articles in this field from researchers of our country motivated us to introduce this subject through this essay. At the end, a description with special regards to the Iranian experience on experimental endometriosis will be produced.

### From “Endometriosis” to “Experimental Endometriosis”

It looks as if the very first academic attempts in the perception of endometriosis occurred through the 17th century. Daniel Christianus Schrönof Jena is assumed to be the first one who made an official dissertation about a disease not dissimilar to what today is known as endometriosis in the *Disputatio Inauguralis Medica de Ulceribus Uteri*. Of note, some aspects of the disease he described cannot be matched with the disease; that is why some references perceive it as endometriosis doubtfully.<sup>1,7-9</sup>

The other famous literature which has been assumed to target this disease is *Dissertatio Medica Inauguralis de Utero Inflammatione Ejusdem* by the Scottish authors Balfour and Smellie in 1776.<sup>1,9</sup>

However, the manuscript of Carl von Rokitansky of Vienna published in 1860 has been attributed to the discovery of endometriosis by some.<sup>10</sup> Though there are discrepancies between his definition and the today definition of the disease, which have made some authors not to believe him as the discoverer of endometriosis,<sup>11</sup> his studies on this subject should be regarded as the start of the dynamic movement about apprehension of endometriosis, which still goes on.

William Russell's contributions in this subject mainly consist of his manuscript in *Johns Hopkins Hospital Bulletin* in 1899.<sup>12</sup> He had probably described what is now known as endometrioma. Richard Te Linde knew him as the man who described endometriosis.<sup>11,13</sup> Russell believed the condition he had described is related to Mullerian duct abnormalities.<sup>12</sup> Thomas Cullen, whether to be known as the discoverer or not, should be appreciated as the one who academically described adenomyosis pathologically and clinically, in his book *Adenomyoma of the Uterus* in 1908.<sup>7,8,10</sup> Friedrich von Recklinghausen's works, especially his theory about the Wolffian origin of the disease, and Iwanoff and Robert Meyer's which concluded in the coelomic metaplasia theory, alongside the studies of Cuthbert Lockyer and De Witt Casler, all lightened up the road for John A Sampson to declare his challenging theories.<sup>10</sup>

In the year 1921, Sampson stipulated that the subjected lesions – today known as endometriosis – are products of peritoneal implantation from perforating hemorrhagic (chocolate) cysts of the ovary, as his first theory.<sup>14</sup> This presentation has been described as an epoch-making one by remarkable authorities such as Emil Novak and some others.<sup>15,17</sup>

Six years later, in 1927, following more comprehensive research he declared the second part of his theory.

Technically, he developed a new conception on the matter of the pathogenesis of the disease: the transtubal retrograde menstruation.<sup>18</sup> In the same year he had described the possibility of metastasis or embolism of endometrial particles through the venous system; which had been previously presumed to be thymatics, by Halban and him.<sup>19</sup>

Sampson's theories, while logically seemed reasonable, needed proof; metaplasia, the phenomenon which was basis of the opposite theory, had been observed in different cells by pathologists. Through the first theory to the second one, a colleague in the same medical school of Sampson's, Victor Jacobson, published a manuscript which endorsed the first theory. Jacobson successfully autotransplanted endometrial tissue in the rabbit in 1922.<sup>6</sup> It was the first model of experimental endometriosis, which also could seal the Sampson's theory on peritoneal implantation.

### Classical Experimental Endometriosis: From Jacobson to Vernon

The first experiments about the transplantation of the uterus may date back to the 19th century. Goldzieher, a contemporary of Rokitansky, due to his attempts for transplantation of the endometrium in the year 1874, is the pioneer in this field.<sup>20</sup> This subject had been almost forgotten until the first years of the 20th century. A manuscript by H. Stilling entitled *Die Entwicklung transplantierter Gewebsteile* in 1903 and another one in 1910 more clarified the subject.<sup>21,22</sup>

Unfortunately, the authors of this essay could not get access to the manuscripts, which are written in German; however, some investigators had studied the one in 1903: “Pieces of uterine and vaginal tissue collected from immature rabbits would continue to grow when transplanted to the spleen of the same animal”,<sup>23</sup> and also: “Stilling was able consistently to implant pieces of vagina, uterus, and endometrium into the spleen and produce cysts of various sizes which were lined with ciliated columnar epithelium. These cysts contained secretion under tension, yet the epithelium often piled up in papillary outgrowths. The wall of these cysts would also regenerate, after a piece had been removed for section.”<sup>24</sup>

Uterine transplantation has perhaps gotten influence from the prior studies about the transplantation of the ovary in the Europe. In 1863, for the first time, report of the allograft of the ovary in the rabbit was made by Paul Bert in Paris. Notwithstanding the unsatisfying results he had, the first successful transplantation of the ovary was performed by Robert Morris in 1895, which was an autograft in New York. Interestingly, his surgery was on a woman. The next year, a successful autograft of the ovary in the rabbit was recorded by Knauer.<sup>25,26</sup> Prior to Stilling, other 9 animal and 2 human reports of the transplantation of the ovary were recorded, which just one of them was a failure.<sup>26</sup>

In 1918, Hesselberg et al studied on the transplantation of the uterus in the guinea pig. Literally, that was one part through her animal series of assessing the quality of autografts and allografts of the different organs. She and her colleagues auto-transplanted some pieces of the uterus in pockets of ear, or allo-transplanted them subcutaneously;

either into the ear, or the abdominal wall. This study, indeed, lacks a clear method section and the exact way grafts had been made is missed. By her descriptions, it seems that she had induced endometriosis successfully; especially in the autografts, with the desired cystic structure, epithelial lining, glands and connective tissue.<sup>27</sup>

Although Goldzieher, Stilling and Hesselberg did valuable studies; the authors believe that credit for the introduction of the endometrial tissue transplantation as the experimental endometriosis is due to Victor Jacobson of Albany.

In 1922, just as the Sampson's Peritoneal implantation theory had spared warmth to the gynecology and pathology societies, Jacobson reported 5 different approaches to create endometriotic lesions in the rabbit, in order to legitimize Sampson's theory. His methods include: (1) Four centimeters of the right horn, at about 5 cm distal to the fimbria was excised, opened longitudinally and scraped. The remained tissue was cut into less than 1 mm particles. The visceral peritoneum got cicatrices. The scrapings were rubbed into the cicatrices and the particles were set into the pelvis. Some blood was released into the cavity. (2) The endometrium of the excised horn got dissected. Two pieces, approximately 1 mm or less, were inserted into the stabbed right ovary at 2 sites of the anterior; one was put under the germinal epithelium. They were stitched with the mesentery over them. (3) The 2 mm-sized endometrial particles of the excised left horn were set under the anterior serous layer of the fundus via 3 stab wounds made by an eye knife. The left mesosalpinx covered the lesion and was stitched to it. (4) The subtle particles of endometrium of the excised segments of left and right horn were set into the 2 pockets with the department of one centimeter of the right mesosalpinx of a pregnant rabbit. (5) A longitudinal incision at the proximal portion of the left horn, anteriorly, in a pregnant rabbit was the only intervention.

Consequently, he concluded that all his methods can result in formation of endometriosis; even the results in pregnancy were more magnificent. At the end, he proposed this rabbit model with advantages of abundant fat in the mesosalpinx; suitable as the implantation site, and the disadvantages of peristalsis; which can be corrected by suturing of the implants, and absence of menopause; which the monkey model sounds more proper. He also highlighted the possible role of cicatrices in the pathogenesis of the lesions.<sup>6</sup>

Jacobson strengthened the knowledge on this matter in the coming years. The next year he published a short article,<sup>28</sup> but more novel was his publishing in 1926 which introduced the monkey model for experimental endometriosis.<sup>29</sup>

Till that time the advantage of the monkey was only the menstruation cycle. A few years later a report of spontaneous endometriosis was made; making endometriosis a common disease between the monkey and human beings.<sup>15,30,31</sup>

The dog as a model for experimental endometriosis was introduced in the year 1927 by O'Keefe and Crossen.<sup>32</sup> At the appendix of 5 dogs, out of ten, endometriosis was developed.<sup>24</sup>

The next 2 decades were almost fruitful years for experimental endometriosis. The studies of Allen, Harbitz,

Weinstein et al, Hobbs & Bortnick, Katz & Szene, Schochet and Hartman are more famous examples.<sup>20,22,33-37</sup>

Allen and Bauer, following Schochet studies in 1916 which showed impossibility of metaplasia of the ovarian epithelium or peritoneum into endometriosis in the anterior chamber of the eye, performed an experiment on the transplantation of endometrium of the rabbit into the anterior chamber of the eye which concluded in cellular growth; however, endometriosis formation should be suspected since they did not report the proliferation of stroma.<sup>24</sup> Allen repeated almost the same study as his previous one in 1932, with a control group. He dissected small particles of endometrium, ovary and peritoneum of the rabbit and injected endometrium alone into one anterior chamber and the others into the other anterior chamber of the rabbit. He reported successful formation of endometriosis consisting of epithelial layer and stroma in a cystic structure during 2 weeks to thirteen months. Of note, he also reported angiogenesis within 24 hours.<sup>37</sup>

Meanwhile, the attempts of Heim et al worth reminding; they did 3 short trials: (1) distribution of the menstrual contents of the monkey in the abdomen, (2) Setting the menstrual contents in the peritoneal cavity with leaving the open fundectomized menstruating uterus in the pelvis, (3) Placing human menstrual contents into the abdominopelvic cavity of the monkey. They concluded no way would result in endometriosis. To challenge their experiments, in 1953 Scott, Te Linde and Wharton did the fundectomy surgery in a monkey and let her live 2 and a half year. The autopsy revealed extensive endometriosis.<sup>16</sup>

Again the endometrial transplantation in the anterior chamber of the eye, which has tight junctions; limiting the immune system and rejection of allografts, was performed, this time in the monkey in 1940 by Markee.<sup>38</sup>

Administration of drugs to the animals with experimental endometriosis may have started with the multiple studies of Hagino; a pioneer in this subject. Administration of diethyl stilbestrol dipropionate in 2 groups of rabbits which received 1 125 000 IU and 975 000 IU, on a basis of 12 500 IU per day, included remarkable growth of the implants in the former.<sup>20</sup>

A novel technique in the induction of endometriosis was created by Hobbs and Bortnick; particles of endometrial tissue were dissected from the underlying layers and injected into the ear vein via syringe. Following pre- and post-operative administration of estrogen, endometriosis of lung parenchyma was induced in the rabbit. However the rate of success was almost low; 8 out of 12 showed some structures of endometrial tissue and just 3 showed pathologically approved endometriosis.<sup>39</sup> The successful induction of endometriosis following implantation in the skin (in the rabbit) can be considered as the achievement of the late 40 seconds.<sup>40</sup>

In 1950, Te Linde and Scott published a praiseworthy study challenging the 2 main theories of the pathogenesis on that time: the Sampson's theory and the coelomic theory; which was highly supported by a prominent gynecologist Emil Novak.

They dissected the endometrium from the underlying layers and cut it into 2-3 mm particles, in pregnant and non-pregnant *Macacusrhesus* monkey. The particles were stitched to the different sites of the previously scarified peritoneum, ovaries and broad ligaments. Compressing the proximal part of the ovarian vein, 2-minute particles were injected into it with some amounts of saline. The newsworthy results of this study were the unsuccessfulness of the injection method and independency of the implants from exogenous estrogen, phase of cycle and pregnancy. As expected, they observed successful endometriosis in 6 out of 7 monkeys.<sup>15</sup>

They later assessed the ability of the menstrual contents to form endometriosis, as Te Linde quotes “histologic viability does not mean physiologic viability.” During laparotomy, the cervix was separated from the uterus, after clamping the lower branches of the uterine arteries. The stump of the cervix was closed and peritonized. The free end of the uterus was allowed to menstruate through the abdominopelvic cavity or the abdominal wall; it was either turned up or aside or sutured to the anterior abdominal wall. They insisted on saving the endometrium intact.<sup>13</sup> These experiments resulted in successful endometriosis formation in 5 of the monkeys; later another paper corrected it as 6, claiming the one with uterus in the abdominal wall also resulted in endometriosis.<sup>16</sup> In further studies in 1953, additional 3 monkeys were subjected to the uterus-to-abdominal wall anastomosis and in all cases experimental endometriosis were confirmed. Hence, the authors highlighted the hypothesis that pelvic peritoneum is not an essential etiologic factor.<sup>16</sup> Te Linde et al concluded that probably in these experiments, the disease was formed by the Sampson’s theory. Although the possibility of what is today known as the induction theory cannot be certainly ruled out.<sup>13,15,16</sup>

The study in 1950 was named as “the first major experimental study of endometriosis in a menstruating animal” by Edward Allen. Maybe, in this study, the most misleading parameter in order to confirm the Sampson’s theory against the coelomic theory which was pointed out by Emil Novak, was the long time needed for formation of endometriosis; almost 3 years for some cases.<sup>15</sup>

In 1953, following Novak suggestion, Scott, Te Linde and Wharton designed an experiment to assess whether blood, by itself, is a potential agent in producing experimental endometriosis out of peritoneum or not. In 4 monkeys their own venous blood was intraperitoneally injected at the first days of cycle. After more than 2 years no pathologic change was observed. The possibility of transformation of the cervical or endosalpingial tissue grafts to endometriosis was rejected in that study.<sup>16</sup>

The aforementioned studies by Te Linde and colleagues, although were distinguished studies which spared the gynecologists remarkable perceptions on endometriosis, lacked proper control groups to be considered a well-structured research.

In a longstanding research by Allen et al in the next year thirteen *Rhesus* monkeys were employed, in which their cervixes just at the transition site of the external os to the vagina were set free and implanted beneath the fascia of

the anterior abdominal wall. They also employed a small number of the monkeys as control group. Performing explorative laparotomies at every 6 months, the follow-up almost continued up to 8 years. With a success rate of two thirds, any effectiveness of administered estrogen and progesterone was denied. The authors of that study opined that the successfulness of the autograft experimental endometriosis had been stabilized enough on that time and the only approaches to investigate the etiopathophysiology of endometriosis were experimental animal study or tissue culture.<sup>41</sup>

A very unconventional, however with remarkable results, was the survey of Ridley and Edwards in 1958, which used the human as model for experimental endometriosis. They enrolled 8 women; 7 African-American and one white. At the first day of their menstruation, a condom secured to a short Colvin cannula which had been inserted into the cervix, was supposed to collect the shedding content while the subject had been put into Fowler’s position in the bed for 12 hours. Hence, the content would include all the naturally shed material without any direct manipulation or trauma to endometrium. After centrifuging, 1.5-2 mL of the content was injected one finger breadth over the symphysis pubis in the midline. After 90-180 days, laparotomies were performed for other reasons and meanwhile, biopsies were taken for the experiment. Out of 8 cases, endometriosis was confirmed in one and was suspicious in another one. The involved subject was a white woman in her twenties, while the others were African-Americans in their thirties or forties.<sup>42</sup>

The use of the rhesus monkey as a model for experimental endometriosis was advanced into a new era in the forthcoming years; through more practical studies by Scott and Wharton, the monkey model was utilized as a model for assessment of pharmacologic agents.<sup>43</sup>

After the preliminary studies on the dog in the 20 seconds, their re-utilization occurred in the 60 seconds, in an almost fascinating approach; pericardial endometriosis. The dogs after receiving 1.5 mg/day DES, underwent hysterectomies. The dissected endometrium, after the abrasion of the pericardium with dry gauze, was put in the pericardial cavity. The administration of DES continued postoperatively. The results were in favor of a new, acceptable method for induction of endometriosis.<sup>32</sup>

The oncoming years included more research on the experimental endometriosis which profited more established manuals of how the experimental endometriosis should be induced. A book by Daniel and Boyle in 1978 dedicated one chapter to the surgical induction of endometriosis.<sup>44</sup> In the same year, Te Linde published a short article on the background of his experience on the subject.<sup>11</sup>

Till that time, most of the efforts had been concerned to prove or disprove the disputes of the pathogenesis. After this time, the studies mostly concern about the different aspects and characteristics of endometriotic lesions, fertility-related issues or, often, the application of various drugs on them.<sup>43,45</sup>

Schenken and Asch in order to assess some of the effects of the fertility-related issues –perhaps the first one in this

matter- performed experimental endometriosis in the rabbit in 1980. Six and 3 days prior to the induction intervention and also every 4 days after that, they administered estradiol cypionate 30 µg/kg IM to each rabbit to produce endometrial hypertrophy. The uterine horns were not left free and end-to-end anastomoses were performed. They only implanted the endometrial tissue, after dissection from its underlying layers. The reported rate of success was 100%.<sup>45</sup>

Inducing endometriosis in the ear of the rabbit through making pockets between the skin and the underlying tissue and insertion of the endometrial particles in the pockets was not an invention of the 80 seconds; however, reminded the investigators of a simple technique, which is almost regardless of operation.<sup>46</sup>

The seminal study of Vernon and Wilson opened a new avenue in the subject. They utilized the lab rat as model for experimental endometriosis. They believe that although the thought had been previously opined by Daniel and Boyle in their book; however, that was the former which materialized the thought.<sup>23</sup> Nonetheless Vernon and Wilson acclaim to be the first investigators who introduced the rat for experimental endometriosis, Schochet mentions the rat model in earlier surveys in the year 1929.<sup>22</sup> Moreover, Golan and colleagues, and Jones used rat for experimental endometriosis a year prior to the publication of Vernon and Wilson, with almost the same methodology.<sup>47,48</sup> Noteworthy, Jones did separate the myometrium from endometrium, while Vernon and Wilson did not. The main reason which has privileged this study over the rest is the well-structured, controlled methodology that concludes one method is the successful technique for induction of endometriosis. The rat have many advantages, alongside their many disadvantages off course, which at last have made them rational choices for investigators who cannot access or afford the monkey.

They used forty-six, 70-day-old Sprague Dawley rats. Their estrous cycles were monitored by taking daily vaginal smears for one month. At the morning of proestrus, they performed the surgeries. The first group consisted of the rats which received 3 implants on the mesentery and one on the utero-ovarian ligament. The implants had the 3 layers, while the serous layer was in contact with the peritoneum and were sutured near the mesenteric blood vessels. The rats of the second group received shower of intra-uterine irrigation on their peritoneal cavity. The third group received shower of saline containing scrapings obtained from loop harvesting of the inner side of the uterus. The fourth group was the sham group. They concluded that the first approach is the only acceptable method and the implants grow up to the 60th day and can be supposed viable up to 10 months after the surgery. Moreover, the mesenteric transplantations seem to be larger and the growth is irrespective of the stage of the reproductive cycle.<sup>23</sup>

This study can be truly regarded as the renaissance of the experimental endometriosis in the literature. Indeed, the following years faced a voluminous number of papers challenging the enigmatic pathophysiology or therapy of endometriosis, based on the established approaches introduced in prior studies. Meanwhile, not only has this study served as a tenacious reference to many investigators,

but also it can be considered as a milestone in the subject.

### The Rat in More Recent Studies

Almost most of the studies on experimental endometriosis in the rat, after the preliminary study of Vernon and Wilson, conform to their protocols, and their methodologies do not differ considerably.<sup>49-53</sup> Perhaps, a recent innovation is the application of fibrin glue for transplantation; although, the quality of the implants may differ significantly from the conventional suturing.<sup>54</sup>

### The Mice: Modern Trends in Experimental Endometriosis

The study of Zamah et al introduced the nude mice as a model for experimental endometriosis. Briefly, they prepared inoculums out of human endometrium and endometriotic tissues. Firstly, the specimens were dissected out of human tissues. They were maintained in cold, sterile Dulbecco's phosphate-buffered saline, with 200 U/mL of penicillin and 200 µg/mL of streptomycin and 20 U/mL of nystatin. Then, they were minced into 1 mm<sup>3</sup> and rinsed with phosphate-buffered saline for 2 times in order to remove cell debris. Each 0.2 mL of the fragmented tissue was mixed with 0.2 mL of phosphate-buffered saline, and 0.4 mL solution was prepared for inoculation. The inoculums were injected subcutaneously or intraperitoneally; between the 2 scapulas, on the dorsal midline, or caudal to the umbilicus, on the ventral midline, respectively, with tuberculin syringes, gauge 18. The mice received 30 µg/kg estradiol cypionate per week, IM. They concluded that both ways results in formation of experimental endometriosis; the subcutaneous method has the benefit of easy accessible implants, while the place of the other one is closer to the actual endometriosis. At the end, they denied single cell suspension capability for formation of endometriosis.<sup>55</sup>

Nisolle et al made a peritoneal pouch during laparotomy, instead of injection, which resulted in 87% rate of success, while in the study of Zamah et al, it was 100%.<sup>56</sup>

The nude mice are athymic. Thus, the process of maturation of T cells is almost incomplete, and consequently, the cellular immunity in these animals is impaired. That is why they are good choices for transplantation of human tissues. They almost act like live medium cultures.<sup>52,53</sup> The other advantage is that the complications of hysterotomies are almost avoided.

Later, severe combined immuno deficient (SCID) mice were successfully introduced as models for experimental endometriosis. This animal lacks both B and T cells.<sup>57,58</sup>

The enthusiasm for introducing new models for experimental endometriosis led the researchers to validate the non-obese diabetic SCID mice (NOD-SCID) as another model. It seems that these NOD-SCID murine models are better choices for longer term experiments exceeding 3 weeks. Grümmer et al maintained the human tissue fragments in the culture medium fortified with 10<sup>-9</sup> mol/L of estradiol-17β for 24 hours.<sup>59</sup>

Human tissues were subcutaneously implanted in the pockets made through less than 3 mm midline ventral incision in NOD-SCID mice; ideal results were concluded.<sup>60</sup>

The allograft models in mice have been proposed elsewhere.

In these methods, one BALB/C mouse is sacrificed and both uterine horns are removed and processed, so the very small fragments are made out of them and at last, they are injected into the peritoneal cavity of 2 mice.<sup>61,62</sup>

TCDD gavage, a chemical substance, has been also proposed to promote the experimental endometriosis in the rodent.<sup>63,64</sup>

As the field of experimental endometriosis developed, more complicated and interesting ways for inducing experimental endometriosis were introduced. Greenberg and Slayden, in a novel approach, validated the transgenic RAG-2/ $\gamma$ (c)KO mice, which plus the dearth of B and T cells, lack Natural Killer cells, too. Their xenotransplantation method included the incubated and processed human endometriosis tissue insertion into the subcutaneous pockets on the abdomen.<sup>65</sup> Since the methods in these studies are almost the same as the aforementioned approaches, the authors decided not to repeat all the details and just to emphasize on the new aspects of methodologies.

Adenomyosis is a disease which is integrated with endometriosis in so many aspects. A recent update by Greaves and White, beautifully describes the different approaches for experimental adenomyosis. Therefore, any further discussion on this subject is referred to their review.<sup>66</sup>

### The Baboon: The Choice Animal Model for Experimental Endometriosis

The Baboon, because of belonging to the order Primates, shares many similarities with the anatomy, physiology and pathology of the human genital system; including menstruation and spontaneous endometriosis. Thus, it is known to be the choice animal model for studying endometriosis. However, since on one side, the authors of this study has limitations to access these animal, and on the other side, their implications have largely been discussed in some review articles by others, this article refers its readers to those papers only.<sup>50,67-70</sup>

### Iranian Experience

Although globally there is an almost a hundred years of experience with experimental endometriosis, Iranian experience is totally new. Most studies have used the rat for their animal model and the auto-transplantation of Vernon and Wilson as their method of induction. The enigmatic nature of endometriosis along with its high prevalence among women, the availability of rats and mice, and the low cost of its materials, all together have made this method a very cheap, practical, feasible, realistic and useful way to study endometriosis. It is high time the Iranian centers became more experienced with experimental endometriosis.<sup>71-76</sup>

### Authors' Contribution

The authors contributed to the conception and design of the manuscript, data collection, writing and approval of the final version.

### Conflict of Interest Disclosures

The authors have no conflicts of interest.

### Ethical Statement

Not applicable

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