Contraception xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

Contraception

journal homepage: www.elsevier.com/locate/con

IF=3.375-胡世福-华中科技大学，兔
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Original research article

Effect of a novel copper-containing intrauterine device material on the endometrial environment in rabbits

Shifu Hu [a,1], Yingying Wang [a,1], Dandan Ke [a], Fang Zhou [a], Guiping Cheng [a], Heyu Liu [a], Wei Xia [a,b,*], Changhong Zhu [a,b,**]

[a] Family Planning Research Institute, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei 430030, China
[b] Reproductive Medicine Center, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei 430030, China

ARTICLE INFO

Article history:
Received 2 February 2018
Received in revised form 5 June 2018
Accepted 11 June 2018
Available online xxxx

Keywords:
Cu/LDPE/MVQ microcomposite
IUD
Contraceptive effectiveness
Adverse effects
Endometrium
Rabbits

ABSTRACT

Objective(s): This study aimed to determine whether intrauterine placement of a novel composite material [copper (Cu) microparticles, low-density polyethylene, and methyl vinyl silicone rubber (Cu/LDPE/MVQ)] could prevent pregnancy in rabbits, and to evaluate the effects of Cu/LDPE/MVQ on the endometrial environment.
Study Design: Eighty sexually mature female rabbits were randomly divided into four groups ($n=20$ each group): control (sham-operated), LDPE/MVQ, Cu/LDPE/MVQ microcomposite, and bare Cu. Ten rabbits from each implant-bearing group were randomly selected for a mating experiment beginning 30 days after insertion. Pregnancy outcomes were observed 15 days after mating. Factors associated with endometrial bleeding and inflammation in the remaining rabbits in each group, and the surface conditions of the implants, were investigated 90 days post-insertion.
Results: The Cu (0 embryo) and Cu/LDPE/MVQ (0 embryo) groups had significantly fewer embryos than the LDPE/MVQ (1.0 ± 0.6 embryos, $p<.05$) and sham-operated groups (4.1 ± 1.3 embryos, $p<.05$). Compared with bare Cu, the Cu/LDPE/MVQ composite material was associated with considerable reductions in injuries and factors associated with abnormal endometrial bleeding and inflammation, such as matrix metalloproteinase 9 (MMP9) and prostaglandin E2 (PGE_2). Additionally, the surface of implanted Cu/LDPE/MVQ remained much smoother than that of implanted bare Cu.
Conclusion(s): This novel Cu-containing intrauterine device material exhibits a similar effect in prevention of pregnancy with bare copper, and lower levels of inflammatory markers.
Implications: This study demonstrates the potential of the novel Cu/LDPE/MVQ microcomposite material as a future substitute for conventional intrauterine device materials.

1. Introduction

The copper intrauterine device (Cu-IUD) is the most extensively used long-acting reversible contraceptive worldwide [1, 2]. A growing body of evidence suggests the appearance of a Cu ion "burst release" within the first few months after Cu-IUD insertion, followed by a gradual decrease and stabilization of Cu ion release [3, 4]. This large release of Cu ions into the uterine cavity is thought to correlate with the adverse effects of Cu-IUDs, such as menorrhagia, intermenstrual bleeding and spotting. Furthermore, constant corrosion leads to the deposit of various products on the surfaces of Cu-IUDs, which may also contribute to side effects [5, 6]. Therefore, although the conventional Cu-IUD achieves a desirable contraceptive efficacy among women of reproductive age, continued use of the device is complicated by numerous side effects, including intermenstrual bleeding, pelvic pain, menorrhagia, and uterine perforation.

Polymer matrix composites with superior abilities to control the release of copper ions have been developed in an attempt to eliminate the disadvantages of the conventional Cu-IUD [7, 8]. Low-density polyethylene (LDPE) and methyl vinyl silicone rubber (MVQ) have been extensively applied in various biomedical contexts because of their excellent biocompatibilities and mechanical properties [9–11]. MVQ is widely used in orthopedic and cardiovascular devices [12], while LDPE has been included in polymer matrix composites, such as the Cu/LDPE nanocomposite IUDs described in our previous studies [13–15]. However, these earlier IUDs did not resolve the aforementioned disadvantages of Cu-IUDs [16]. Therefore, to overcome the disadvantages of conventional Cu-IUD materials, in this study we evaluated the usefulness of a Cu/LDPE/MVQ microcomposite developed in our laboratory as a novel IUD material [17]. Despite previous analyses, however, it

* Corresponding author.
** Corresponding author. Tel.: +86 27 83692651; fax: +86 27 83692651.
E-mail address: reprodcentre@163.com (C. Zhu).
[1] These authors contribute equally to this work.

https://doi.org/10.1016/j.contraception.2018.06.002
0010-7824/

remained unclear whether this novel Cu/LDPE/MVQ microcomposite meets the requirements for a contraceptive device and whether this material adversely affects the endometrial environment.

Therefore, the aims of this study were to determine whether intrauterine placement of the novel composite material Cu/LDPE/MVQ could prevent pregnancy in rabbits, and to evaluate the effects of Cu/LDPE/MVQ on the endometrial environment by measuring the expression of inflammatory markers, such as matrix metalloproteinase 9 (MMP9) and prostaglandin E2 (PGE_2), and factors associated with endometrial bleeding, such as angiopoietin-2 (ANG2), tissue plasminogen activator (t-PA), and CD34.

2. Materials and methods

2.1. Materials

The IUD component materials were formed by the Department of Materials Science and Engineering of Huazhong University of Science and Technology. Briefly, a Cu microparticle (micro-Cu)/LDPE/MVQ composite was constructed using physicochemical methods. Using a melt-blending process, the MVQ and LDPE powders were combined with high-quality micro-Cu (15% weight) in a single-screw extruder at a screw speed of 20–25 rpm. The extruder was maintained at temperatures of 115°C, 140°C, and 155°C from the hopper to the die, respectively. The composite material contained uniformly distributed micro-Cu within a framework comprising the LDPE/MVQ matrix. The spacing within the matrix provides osmotic pathways for Cu ions and corrosion mediation. Accordingly, the corrosion rate and Cu ion release velocity are effectively controlled by the separation of micro-Cu within the matrix. Three different materials were formulated: micro-Cu/LDPE/MVQ, LDPE/MVQ, and bare copper (Cu).

2.2. Animals and treatment

Sexually mature female and male rabbits weighing 2.5–3.0 and 3.0–4.0 kg, respectively, were purchased from the experimental animal center of Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China. The rabbits were maintained under controlled conditions (12-h light/dark photoperiod; temperature: 23±2°C, 50±10% relative humidity) and allowed access to food and tap water ad libitum. The rabbits were acclimated to this environment for 5 days prior to the experiments. All of the animal experiments in this study were performed according to the National Institutes of Health Guiding Principles in the Care and Use of Animals, and the protocols were approved by the Reproductive Medicine Review Board of Tongji Medical College, Huazhong University of Science and Technology.

Eighty sexually mature female rabbits were randomly assigned to four experimental groups (n=20/group): sham-operated (SO) control, bare Cu (Cu), LDPE/MVQ, and Cu/LDPE/MVQ; the latter three groups were used to evaluate the contraceptive effects and safety of the implanted bare Cu and microcomposites, respectively. Before insertion, rabbits in the Cu, LDPE/MVQ, and Cu/LDPE/MVQ groups were anesthetized, and then the designated material was inserted into the caudal portion of the right uterine horn and secured to the uterine wall with sutures via laparotomy and uterotomy. The contralateral horn was left untreated. Rabbits in the SO group underwent the same surgical procedures without the implantation of material into the uterine horn. All implanted materials were approximately 1 mm in diameter and 5.9 mm in length, with an approximate surface area of 20 mm^2.

The rabbits' estrous cycles were monitored daily by vaginal lavage, and a microscopic cellular stage assessment was used to calculate cycle length. Ten rabbits from each material-bearing group were randomly selected for a mating experiment beginning 30 days after insertion of the implant. The selected female rabbits were allowed to cohabit with male rabbits, and successful mating was assessed by a light microscopic examination of vaginal swabs during the mating period. Subsequently, the animals were sacrificed and pregnancy outcomes were observed via uterotomy on day 15.

The remaining rabbits in each material-bearing group were killed via laparotomy on day 90 post-insertion. The uterine tissues were quickly removed, placed on ice, dissected, and frozen at -80°C. The remaining tissues were fixed for histopathological examination.

2.3. Immunohistochemistry (IHC) and Western blotting

The effects of the different implanted materials on MMP9, CD34, and ANG2 proteins in endometrium were analyzed using immunohistochemistry (IHC) and western blotting. The methods are detailed in the supplementary material.

2.4. ELISA

The concentrations of PGE_2 and t-PA in the endometrial tissues of the rabbits were determined as described in a previous report [14]. Briefly, tissue samples were homogenized on ice, centrifuged for 15 min at 4°C (3500 rpm), and examined using commercially available enzyme-linked immunosorbent assay (ELISA) kits (Enzyme-linked Biotechnology Co., Ltd., Shanghai, China) according to the manufacturer's instructions. The absorbances in the ELISA plate wells at 450 nm were measured using a microplate reader. Data were expressed as the means±S.D. of three independent experiments.

2.5. Determination of the surface characteristics of the implanted materials using scanning electron microscopy

Following removal from the rabbits, the surface conditions of the Cu/LDPE/MVQ microcomposite and Cu implants were characterized via scanning electron microscopy (SEM). All samples were gilded before examination. For comparison, the surface features of original (i.e., non-corroded) Cu/LDPE/MVQ microcomposite and Cu materials were also examined using the same process and instrument. At least three replicates of each type of material were evaluated.

2.6. Statistical analysis

Data from repeated experiments were presented as means±S.D.. SPSS (SPSS Inc., Chicago, IL, USA) was used for the statistical analysis. A one-way analysis of variance or chi-square test was used for the statistical evaluation, as appropriate. A $p<.05$ was considered statistically significant.

3. Results

3.1. Fertility trial

Table 1 presents the results of the fertility trial. We detected no embryos in the uterine horns of rabbits fitted with Cu alone or Cu/LDPE/

Table 1
Fertility test of different materials

Group (n=10 each)	No. of embryos in material-bearing uterine horn (mean±S.D.)	No. of embryos in contralateral uterine horn (mean±S.D.)	No. of pregnant animals	Fertility rate (%)
SO	4.1±1.3*	3.8±0.9	10	100
LDPE/MVQ	1.0±0.6#*	4.6±1.3	7	70
Cu/LDPE/MVQ	0#	4.2±1.4	0	0
Cu	0#	3.4±0.8	0	0

SO, sham-operated; LDPE/MVQ, low-density polyethylene/methyl vinyl silicone rubber; Cu/LDPE/MVQ, copper microparticle/low-density polyethylene/methyl vinyl silicone rubber; Cu, copper.
* p<.05 when compared to the Cu group.
\# p<.05 when compared to the SO group.

MVQ, and reduced numbers of embryos in the uterine horns containing the LDPE/MVQ (1.0±0.6 embryos, p<.05) material without copper compared to the control (SO) uterine horns (4.1±1.3 embryos). By contrast, normal embryos were observed in all of the contralateral uterine horns; additionally, no significant differences in the numbers of embryos were observed among the SO (3.8±0.9 embryos), LDPE/MVQ (4.6±1.3 embryos), Cu/LDPE/MVQ (4.2±1.4 embryos) and Cu (3.4±0.8 embryos) groups (p>.05).

3.2. Effects of different materials on endometrial cytokine levels

The effects of the different materials on endometrial levels of PGE_2 and t-PA were evaluated using ELISAs. As shown in Fig. 1, the levels of PGE_2 and t-PA did not differ significantly between the SO and LDPE groups (p>.05). By contrast, both PGE_2 and t-PA levels were significantly increased (p<.05) in the Cu and Cu/LDPE/MVQ groups. Notably, however, PGE_2 and t-PA levels were significantly lower in the Cu/LDPE/MVQ group, compared with the Cu group (p<.05).

3.3. IHC evaluation of MMP9, CD34, and ANG2 expression in the endometrial tissues

Next, IHC was used to evaluate whether the different implanted materials affected the endometrial expression of MMP9, CD34, and ANG2, which are associated with bleeding and inflammation. As shown in Fig. 2, significantly higher levels of MMP9, CD34, and ANG2 were detected in the Cu group, compared to the SO, LDPE/MVQ, and Cu/LDPE/MVQ groups (p<.05). Furthermore, no significant differences in the levels of MMP9 and ANG2 were observed among the latter three groups (p>.05). Notably, significantly higher levels of CD34 were detected in the Cu/LDPE/MVQ group, compared to the SO and LDPE/MVQ groups (p<.05).

3.4. MMP9, CD34, and ANG2 protein expression in the endometrium

To confirm the IHC results, western blotting was used to further evaluate the expression of MMP9, CD34, and ANG2 in endometrial tissues. As shown in Fig. 3, the expression of all three proteins was significantly higher in the Cu group than in the SO, LDPE/MVQ, and Cu/LDPE/MVQ groups (p<.05). The expression of MMP9, CD34 and ANG2 was basically consistent with the IHC results; the latter three groups did not differ significantly in terms of the expression of these inflammatory markers.

3.5. Surface characteristics of the implanted materials

Finally, SEM was used to evaluate the surface characteristics of the corroded implanted materials and corresponding original materials. As shown in Fig. 4, the surface of the implanted Cu/LDPE/MVQ was as

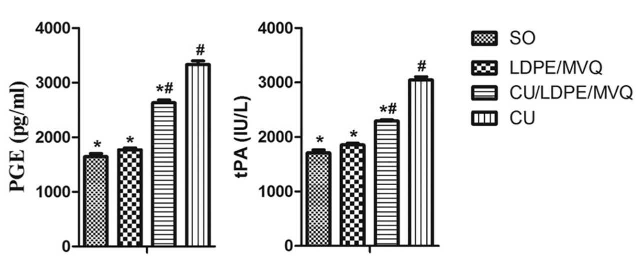

Fig. 1. Comparison of prostaglandin E2 (PGE_2) and tissue plasminogen activator (t-PA) levels in different groups at day 90 post-insertion. Enzyme-linked immunosorbent assays were used to measure the levels of these factors in endometrial tissues from different groups. Data are presented as means±S.D. *p<.05 when compared to the Cu group. #p<.05 when compared to the control (SO) group. SO, sham-operated; LDPE/MVQ, low-density polyethylene/methyl vinyl silicone rubber; Cu/LDPE/MVQ, copper microparticle/low-density polyethylene/methyl vinyl silicone rubber; Cu, copper.

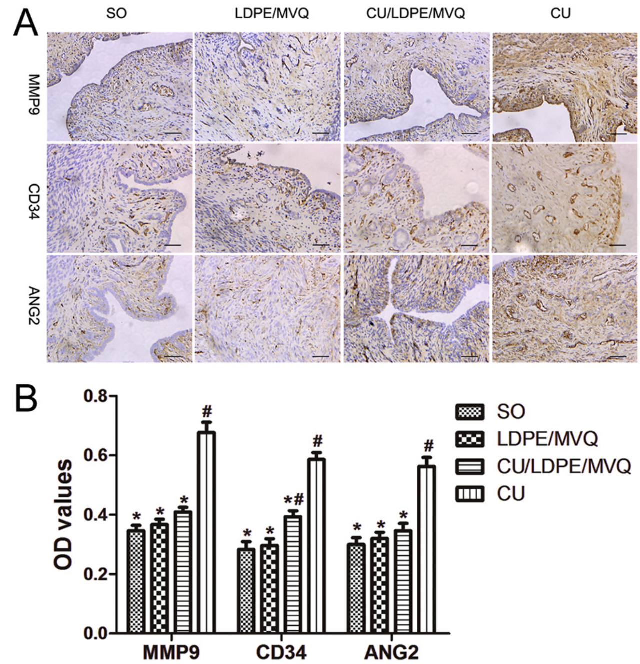

Fig. 2. Immunohistochemical localization and quantification of matrix metalloproteinase 9 (MMP9), angiopoietin 2 (ANG2), and CD34 in the endometrium at 90 days post-insertion. A: Immunohistochemical localization; B: Immunohistochemical quantification using Image J software. Protein expression levels were quantified using optical density (OD) values. Data are presented as means±S.D. *p<.05 when compared to the Cu group. #p<.05 when compared to the SO group. SO, sham-operated; LDPE/MVQ, low-density polyethylene/methyl vinyl silicone rubber; Cu/LDPE/MVQ, copper microparticle/low-density polyethylene/methyl vinyl silicone rubber; Cu, copper. Scale bar=50 μm.

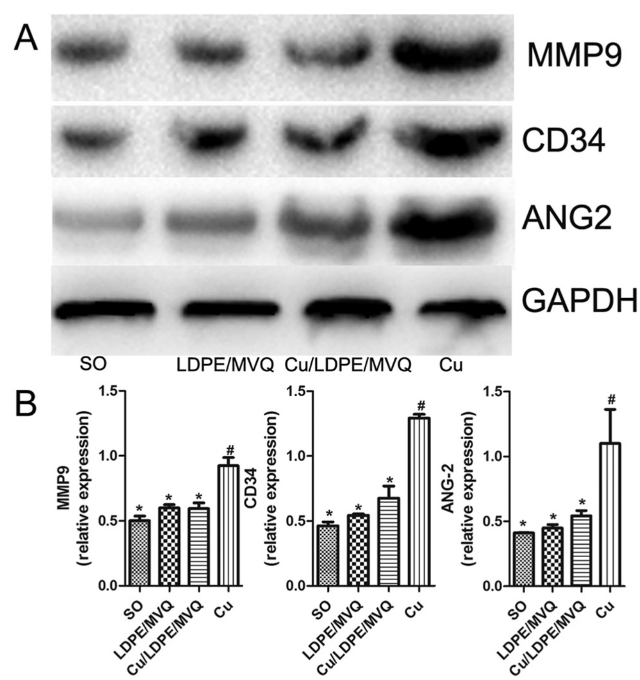

Fig. 3. Western blot analysis and relative protein expression of matrix metalloproteinase 9 (MMP9), CD34, and angiopoietin 2 (ANG2) in the endometrium at 90 days post-insertion. A: Western blot; B: Western blots quantified using Image J software. Data are presented as means±S.D. *p<.05 when compared to the Cu group. #p<.05 when compared to the SO group. SO, sham-operated; LDPE/MVQ, low-density polyethylene/methyl vinyl silicone rubber; Cu/LDPE/MVQ, copper microparticle/low-density polyethylene/methyl vinyl silicone rubber; Cu, copper.

Please cite this article as: Hu S, et al, Effect of a novel copper-containing intrauterine device material on the endometrial environment in rabbits, Contraception (2018), https://doi.org/10.1016/j.contraception.2018.06.002

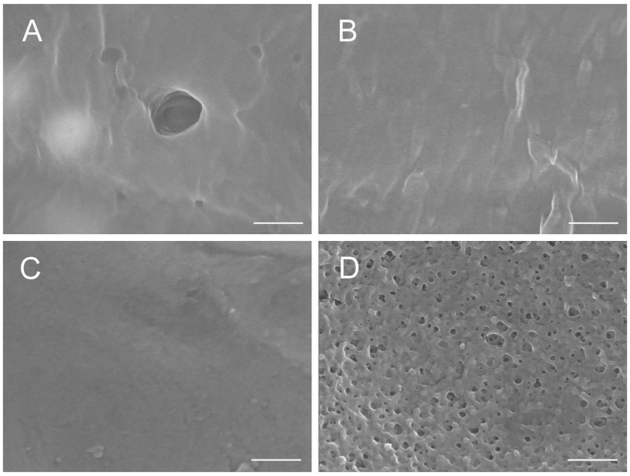

Fig. 4. Surface characteristics of implanted materials. Scanning electron microscope images of (A) original and (B) corroded copper microparticle/low-density polyethylene/methyl vinyl silicone rubber (MVQ) composite (Cu/LDPE/MVQ) and (C) original and (D) corroded bare copper (Cu). Note that the surface of the corroded Cu/LDPE/MVQ microcomposite is almost as smooth as that of the original Cu/LDPE/MVQ microcomposite. By contrast, the corroded Cu has a much rougher surface than the original bare Cu material. Scale bar=500 nm.

smooth as that of the original microcomposite. By contrast, the surface of the implanted bare Cu was corroded and much rougher than that of the original Cu and the corroded Cu/LDPE/MVQ.

4. Discussion

Prior to Cu-IUDs, inert IUDs synthesized from non-medicated materials, such as polyethylene, were used widely throughout the world. However, these IUDs are limited by a poor contraceptive efficacy [18], which may be associated with the single contraceptive mechanism of a sterile inflammatory response within the uterus. This problem was addressed by the addition of Cu, a metal that effectively prevents pregnancy, to the IUD [19, 20]. Currently available Cu-IUDs rely on well-documented and generally accepted contraceptive mechanisms, including the release of Cu by corrosion, mechanical stimulation of the endometrial environment, and intrauterine inflammation [1, 21–23]. However, these conventional Cu-IUDs have many disadvantages, including intermenstrual bleeding, menorrhagia, and pelvic pain [24].

To overcome the aforementioned shortcomings of conventional Cu-IUDs, our team developed novel Cu/LDPE/MVQ microcomposites and evaluated whether these novel materials could achieve the required contraceptive efficacy. In this study, no embryos were observed after 1 month in experimental groups of rabbits that were implanted with Cu/LDPE/MVQ and bare Cu. In other words, these devices achieved contraceptive rates of 100%, which indicated that the Cu/LDPE/MVQ microcomposite yielded an excellent contraceptive effect equal to that of bare Cu. Previous reports have shown that micro-Cu inside a Cu/LDPE composite will corrode to release Cu^{2+} in a simulated uterine solution [25], which may be a mechanism that improves the contraceptive effect of the IUD [20]. A contragestive effect may also be involved in this contraceptive mechanism [26].

Increasing evidence demonstrates that traditional Cu-IUDs can cause endometrial bleeding and inflammation [18, 24]. Furthermore, many studies have indicated that the bleeding is associated with increases in PG expression, fibrinolytic activity, and endometrial angiogenesis [17, 27–31]. PGs are a class of highly bioactive and functional blood vascular regulatory factors. PGE_2 is the primary PG expressed by the endometrium. Many studies have reported significant alterations in PG levels after Cu-IUD insertion [27, 28]. t-PA, a main initiator of fibrinolytic system activation, plays an important role in the maintenance of normal hemokinesis. Excessive fibrinolysis and bleeding may therefore be associated with elevated t-PA production [15]. Furthermore, CD34 is a microvascular endothelial cell marker, and abnormal angiogenesis is considered to be associated with bleeding [29]. ANG2, a member of the angiopoietin family, plays a critical role in angiogenesis [30]. According to Li et al., ANG2 levels increased significantly following exposure to traditional bare Cu in vivo [31]. Interestingly, we observed only slight increases in t-PA, CD34, and ANG2 levels in the Cu/LDPE/MVQ microcomposite group, and the increases in this group were smaller than in the bare Cu group.

Furthermore, a previous study indicated that pain is caused by increased levels of inflammatory factors [32]. MMP9, a type IV collagenase/gelatinase, is often associated with inflammatory reactions [33]. The present findings indicate that the novel Cu/LDPE/MVQ microcomposite had a weaker effect on endometrial MMP9 levels compared with bare Cu.

Among the various factors that may induce endometrial injury in the rabbit model, the surface condition of the Cu-IUD material, in addition to the Cu release rate, may be pivotal. A morphological study illustrated that because of corrosion, the bare Cu surfaces of currently available Cu-IUDs become increasingly coarse over time in a uterine solution [34], and this is associated with the adherence of stiff corrosion products, such as cuorite (Cu_2O), to the corroded surface [16]. Researchers believe that friction between the implanted Cu-IUD and the endometrial interface causes damage to the endometrium. Accordingly, endometrial injury is expected to increase as the surface of the implanted Cu-IUD material coarsens and stiffens [13]. Our results demonstrate that the corroded Cu had a much coarser surface than the corroded Cu/LDPE/MVQ microcomposite. This suggests that the Cu/LDPE/MVQ microcomposite is much less likely to cause injury than bare Cu.

5. Conclusion

In summary, this novel Cu-containing intrauterine device material prevented pregnancy in rabbits as effectively as other copper materials, with a reduction in endometrial inflammation.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (grant no. 81671507, 81771570 and National Key Research and Development Program (grant no. 2016YFC1000903).

Disclosure

The authors report no conflicts of interest in this work.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.contraception.2018.06.002.

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