Published in final edited form as:

Contraception. 2013 March ; 87(3): 380–384. doi:10.1016/j.contraception.2012.08.032.

Feasibility of LNG-IUS in a baboon model

Jason D. Bell^{1,*}, Ingrid L. Bergin², Melissa F. Natavio³, Fatima Jibrel¹, Melissa K. Zochowski¹, William J. Weadock⁴, Scott D. Swanson⁴, David M. Aronoff^{5,6}, and Dorothy L. Patton⁷

¹Program on Women's Healthcare Effectiveness Research, Department of Obstetrics & Gynecology, University of Michigan

²Reproductive Sciences Program, University of Michigan

³Unit for Laboratory Animal Medicine, University of Michigan

⁴Department of Obstetrics & Gynecology, University of Southern California

⁵Department of Radiology, University of Michigan

⁶Division of Infectious Diseases, Department of Internal Medicine, Department of Microbiology & Immunology, University of Michigan

⁷Department of Obstetrics & Gynecology, University of Washington

Abstract

Background—The baboon (*Papio hamadryas anubis*) is an attractive model for intrauterine contraception research due to anatomic similarity to the human. Although non-human primates have previously been used for intrauterine contraception research, it was unknown whether modern intrauterine devices (IUDs) can be placed in an anatomically similar position in the baboon. This study sought to determine whether human-use IUDs could be seated correctly in the uterus of the baboon.

Study Design—The levonorgestrel-releasing intrauterine system (LNG-IUS) was placed ex vivo into two baboon uteri collected at necropsy and in 3 living, reproductively proven baboons.

Results—Correct placement of human-use IUDs in the baboon was confirmed by both MRI and ultrasound. This study establishes that a LNG-IUS can be inserted into the baboon uterus and maintained without clinically adverse effects for at least 6 months. The positioning of the device is similar to positioning found in women.

Conclusion—These findings provide important support for studying IUD safety and efficacy in the baboon.

1. Introduction

Currently, intrauterine devices (IUDs) are among the most effective forms of contraception, with a failure rate of <1%. However, IUD use in the U.S. is significantly lower compared to other countries, with one of the potential reasons being the perceived correlation between IUD use and the occurrence of pelvic inflammatory disease (PID). Due to ethical

Corresponding author: Jason D. Bell MD, MPH, MS, University of Michigan, Department of Obstetrics and Gynecology, 1500 East Medical Center Drive, L4510, Ann Arbor, MI 48109, jasonbel@umich.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

constraints, studying the relationship between infection and IUD use in humans is limited primarily to epidemiological investigations. Although a valuable part of human health research, epidemiological investigations can be subject to confounding factors that are difficult to control. Animal-model challenge studies provide an important platform to investigate this assertion in a prospective manner. However, such studies have been limited by an inability to insert modern human-use IUDs into the uteri of small animals. The use of such devices in larger animals, such as non-human primates (NHP), is an attractive alternative but the ability to place modern human-use IUDs in an anatomically-similar manner in these animals has not been established. Early IUDs, (i.e. Lippes loop, Margulies spiral) have been successfully placed in several NHPs, most commonly macaques. These early studies confirmed placement of the IUD by plain film radiography. Compared to these early NHP models of IUDs, the baboon (*Papio hamadryas anubis*) is a more attractive animal for use because of its similarity to *Homo sapiens* in reproductive anatomy and physiology. As in humans, it has a rectilinear cervical canal, allowing for transcervical IUD insertion. This is unlike the macaque, which has a tortuous cervix requiring surgical insertion. Baboons are larger than macaques, also facilitating placement of IUD without the need for device modification. Earlier studies in macaques used IUDs that had been modified in size. Finally, the prominent cyclical hypertrophy of perivulvar skin (“sex skin”) in baboons is a reliable, non-invasive means of determining cycle stage. Despite these theoretical advantages, appropriate IUD placement and retention must be established experimentally in order to fully utilize a baboon IUD model.

The feasibility of transcervical insertion of modern hormone-releasing IUDs and the position of these IUDs within the baboon uterus has not been compared to humans. The objective of the present study was to determine whether an unmodified levonorgestrel-releasing intrauterine system (LNG-IUS) could be placed and retained in the baboon uterus and whether its intrauterine position as determined by magnetic resonance imaging (MRI) and ultrasound was comparable to that in humans.

2. Materials and methods

2.1. IUDs

Five LNG-IUS devices, each consisting of a 32-mm by 32-mm T-shaped IUD, (Mirena®, Bayer Healthcare Pharmaceuticals Inc., Wayne, NJ) implanted with 52 mg of LNG were purchased for use in this study. Levonorgestrel release from this device is reported at 20 mcg/day in humans (www.mirena-us.com accessed May 17, 2012).

2.2. Ex vivo arm

Approval for use of de-identified patient imaging records was granted by the Institutional Review Board at the University of Michigan Medical School (IRBMED; HUM#00038480).

5.2.1. Animal use and tissue harvesting—Tissues from two female olive baboons (*Papio hamadryas anubis*) from a large research colony Texas Biomedical Research Institute, San Antonio, TX (TBRI), were used. Approval for use of animal subjects was granted by the TBRI Institutional Animal Care and Use Committee. The baboons were 10 and 28 years old and had been euthanized for other studies by intravenous injection of sodium pentobarbital (Vortech Pharmaceuticals, Dearborn, MI). The vagina, cervix, uterus, oviducts, and ovaries were harvested en bloc. Tissues were frozen without fixation at -80°C for 1 or 6 months before shipping overnight on ice. Upon receipt, tissues were kept at -80°C for 1 month until the experiment.

2.2.2. Placement of IUD—Tissues were thawed at 4°C overnight. Placement was performed under BSL-2 conditions. Insertion of the LNG-IUS was conducted according to the manufacturer's instructions using the standard introducing device.

2.2.3. Fixation after device placement—Following LNG-IUS placement, the uteri were fixed in 10% neutral buffered formalin at 10:1 volume ratio for approximately 1 month prior to MR imaging. The uteri were imaged while formalin-immersed within polypropylene containers (Nalgene, Rochester, NY).

2.2.4. MR imaging—MR images were acquired on a 2.0T Unity/Inova system (Varian Associates, Palo Alto, CA). Images were obtained with a multislice, spin-echo pulse sequence with a TR of 10 s, TE of 25 ms, and field-of-view (FOV) of 150 mm. In-plane resolution was 0.5 mm and slice thickness was 1.0 mm. Four averages were obtained for each of 30 slices.

Comparison was made to MR images from women with an LNG-IUS in place obtained from the University of Michigan radiology archive. Images were de-identified; approval from IRBMED was received prior to image review.

2.3. In vivo arm

Approval was received from the TBRI Institutional Animal Care and Use Committee (IACUC) and an exemption for off-site animal work was filed with the University of Michigan Committee for the Care and Use of Animals (UCUCA).

2.3.1. Animal selection—Three reproductively mature female olive baboons from a large research colony (TBRI, San Antonio, TX) were used. The baboons were 6, 10, and 11 years old and had a proven reproductive history of at least one vaginal delivery. Each animal underwent a thorough health check prior to enrollment in the study. This evaluation included a complete physical examination as well as a complete blood count and basic chemistry panel. The perivulvar skin ("sex skin") stage was recorded and two animals were at stage 0 (deflated) and one was a stage 3 (inflating). Turgescence is scored on a scale from 0–4, with 4 representing maximum swelling. Inflating is associated with the follicular (estrogen dominated) phase and deflating is associated with the luteal (progesterone dominated) phase

2.3.2. Anesthesia—Animals were sedated by intramuscular injection of ketamine 10 mg/kg (Ketaset, Fort Dodge, Iowa, USA) and xylazine 0.5 mg/kg (Rompun, Bayer Animal Health). They were intubated and maintained on isoflurane inhalation anesthesia on a rebreathing circuit for the remainder of the procedure. Animals were sedated by the same protocol for follow-up physical examination and transabdominal ultrasound once weekly for 4 weeks and then monthly for 5 months until device removal at 24 weeks (6 months).

2.3.3. Ultrasound imaging—Ultrasound images were obtained immediately following device placement and at follow-up examination using a GE LOGIQ 5 (GE Healthcare, Wauwatosa, WI) and a 5C Curved Array Transducer (3–6.7 MHz). Gel was applied to the suprapubic area and the probe was placed vertically. The uterus was located and the device location was established and images obtained.

2.3.4 LNG-IUS placement: ex vivo—The ex vivo reproductive tract was stabilized by holding the uterine body in a slightly anteverted position, mimicking the natural uterine position in a supine body. Because the cervixes were stiffer than expected, possibly due to post-mortem change or freeze-thaw, a 15 Pratt dilator was necessary for cervical dilation. A uterine sound was used to measure the uterine cavity lengths, which were 4.5 and 4.75 cm,

respectively. After dilation, the LNG-IUS inserter device was used and the system was deployed at the uterine fundus without difficulty. The applicator was discharged and withdrawn, leaving the LNG-IUS in place, with the indicator strings extending through the cervix. The indicator strings and the vagina (up to the external cervical os) were excised following the insertion experiment to allow for imaging.

2.3.5 LNG-IUS placement: *in vivo*—Insertion of the LNG-IUS in live animals was conducted according to the manufacturer's instructions using the standard introducing device. Animals were positioned in ventral recumbency (prone) with the hind limbs folded underneath their abdomen. A pediatric Pederson speculum was used to visualize the vagina and cervix. The cervix was then cleansed with Betadine solution and grasped with a tenaculum. The cervical os was cannulated with a disposable os finder (Cooper Surgical, Trumbull, CT). Each animal was dilated to a #14 Hank dilator. The uterine sound was then used to measure the uterine size. Two animals measured 5 cm and the other measured 5.5 cm. The LNG-IUS inserter device was used and the system was deployed at the uterine fundus. After placement, the strings were trimmed to the level of the cervical os and the tenaculum was removed.

For device removal at 6 months, the same protocol was followed for visualization of the vagina and cervix. The cervix was grasped with a tenaculum and the device removed using a ring forceps in 2 animals and an IUD hook in the other. In all three animals, the removal was completed without difficulty.

3. Results

3.1 Ex vivo and in vivo IUD placement

IUD placement in the ex vivo samples was performed without difficulty. Similarly, transcervical in vivo placement was performed similarly to placement in humans without complications. Details of both procedures are described in the methods.

4.2 Verification of device placement: MR imaging and ultrasound

For the ex vivo samples, the anatomic localization of the LNG-IUS was identified by high resolution MRI. In Fig. 1 the LNG-IUS is fundally situated, with the entire device contained within the uterine cavity, which is the appropriate anatomic placement for this device. The arms of the device were not fully deployed, which occasionally also occurs in humans. The strings were also identifiably protruding from the cervical os. The second uterus (not shown) had similar fundal placement with the entire device also within the uterine cavity. Fig. 2 is an image from a human with an LNG-IUS in place. Similar to the baboon, the LNG-IUS is at the fundus. The arms of the device are completely open, which marks a difference from the baboon (Fig. 1).

For the in vivo portion of the study, transabdominal ultrasound was used to verify LNG-IUS placement, rather than MRI, as ultrasound would be utilized for this purpose in standard clinical practice. The LNG-IUS was positioned at the fundus in all three cases. Hard-copy images of the ultrasound could not be obtained due to limitations of the (field-quality) equipment. Due to ease in verifying correct LNG-IUS placement with transabdominal ultrasound in this animal model, no transvaginal ultrasound was performed. Following confirmation of device placement, animals were monitored for 1 h to ensure the devices were well tolerated and that there was no expulsion in the immediate post-placement period.

Serial sedation and examination was performed at intervals over the next 6 months with cervical exam and ultrasonography to verify proper positioning of the device. All 3 animals retained the devices for the full duration of the study without showing adverse behaviors or

clinical signs. Additionally, during the in vivo study, the baboons showed menstrual cycle alterations (cessation of cycling) and cervical mucus changes similar to that observed in women. Using a standardized turgescence scoring system for monitoring of reproductive cycle phase the baboons remained at perivulvar turgescence of 0 or 1 throughout the study, consistent with the luteal (progesterone-dominated) phase. The devices were removed without incident at the close of the study and the animals were returned to the colony population after physical examination to verify their normal health status.

4. Discussion

This small study establishes that an LNG-IUS can be easily and correctly positioned within the uterus of a reproductively proven baboon, both ex vivo and in vivo. MRI was effective in evaluating positioning in necropsy tissues. In women, this imaging modality can demonstrate the uterus in any position and is particularly useful in locating IUDs when ultrasound images are suboptimal. The straight cervical canal of the baboon made placement similar to that in women. Moreover, the fact that the LNG-IUS was contained completely within the uterus in both the in vivo and ex vivo arms of the experiment is favorable. In an earlier baboon model, extension of an IUD through the cervical canal increased the risk of expulsion (unpublished communication, V.C. Stevens, Ohio State University).

There are several limitations to the current study. First, the device arms did not deploy completely in the ex vivo arm. It is unlikely that this would impact contraception, but it could potentially increase the risk of expulsion in later trials. Anecdotally, IUDs in women are often detected in a non-fundal position with partial deployment of device arms and retain efficacy. Another limitation is that the sample size of this study was small and expulsion rates could not be addressed. Though it was encouraging that the device was not expelled, it would be reasonable to suggest the expulsion rate would be similar to that seen in women (~4%). Lastly, the use of a dilator prior to using the inserter is not something that is always performed in women having the device inserted. This is an anatomical difference between women and baboons, and it could be suggested that this may lead to an increased risk of perforation. Though plausible that a perforation could occur, we used ultrasound to verify fundal placement.

In summary, we demonstrate that the LNG-IUS can be placed in baboons in a manner similar to humans. The device was positioned appropriately, was well tolerated by the animals, and was not expelled in the first six months after placement. The verification of this animal model for IUD utilization is applicable toward future studies of IUD safety and efficacy and infection susceptibility. For example, our group has recently developed a baboon model of *C. trachomatis*-associated PID. The data from this IUD study will be useful in adapting our infection model to the study of the potential association between IUDs and PID.

Acknowledgments

The authors want to thank the University of Michigan Pathology Cores for Animal Research for assistance in the *ex vivo* experiment, and the veterinary and laboratory animal care staff at Texas Biomedical Research Institute for their assistance with the live animal study.

This project was supported by the fellowship in family planning. The first author (J.B.) is funded by the National Institutes of Health K12 HD065257.

References

1. Trussell, J., et al. Contraceptive Technology. 19. New York: Ardent Media Inc; 2007.

2. Hubacher D, Finer LB, Espey E. Renewed interest in intrauterine contraception in the United States: evidence and explanation. *Contraception*. 2011; 83:291–4. [PubMed: 21397085]
3. Meirik O. Intrauterine devices - upper and lower genital tract infections. *Contraception*. 2007; 75(6 Suppl):S41–7. [PubMed: 17531615]
4. Mohllajee AP, Curtis KM, Peterson HB. Does insertion and use of an intrauterine device increase the risk of pelvic inflammatory disease among women with sexually transmitted infection? A systematic review. *Contraception*. 2006; 7:145–53. [PubMed: 16413845]
5. Grimes DA. Intrauterine device and upper-genital-tract infection. *Lancet*. 2000; 356:1013–9. [PubMed: 11041414]
6. Doyle LL, Chandler G. *Erythrocebus patas* - Anatomically suitable primate model for reproductive studies. *Fertil Steril*. 1973; 24:648–51. [PubMed: 4198099]
7. Breed WG, et al. Effect of an intrauterine device on menstrual cyclicity and luteal function in baboon. *J Reprod Fertil*. 1972; 28:249. [PubMed: 4621664]
8. Chai D, et al. Olive baboon (*Papio anubis anubis*) as a model for intrauterine research. *J Med Primatol*. 2007; 36:365–9. [PubMed: 17976041]
9. Doyle LL, Harrison RM. Studies on norgestrel-releasing intrauterine devices in *Erythrocebus patas* monkeys. *Int J Fertil*. 1981; 26:112–5. [PubMed: 6114061]
10. Bell JD, et al. Nonhuman primate models used to study pelvic inflammatory disease Caused by *Chlamydia trachomatis*. *Infect Dis Obstet Gynecol*. 2011:675360. [PubMed: 21869858]
11. Shaw ST, et al. Influences of Copper and Design of Intrauterine-Devices on Endometrial Bleeding in Monkeys. *Contraception*. 1976; 14:9–15. [PubMed: 824096]
12. Chai D, et al. Olive baboon (*Papio anubis anubis*) as a model for intrauterine research. *J Med Primatol*. 2007; 36:365–9. [PubMed: 17976041]
13. Mirena, Mirena Package Insert. Bayer Healthcare Pharmaceuticals Inc; Wayne, NJ: 2009.
14. Honore, EK. The Baboon in Biomedical Research. VandeBerg, W-B.; Tardiff, editors. Springer; 2009.
15. Mirena, Mirena Package Insert. Bayer Healthcare Pharmaceuticals Inc; 2009.
16. Mark AS, Hricak H. Intrauterine contraceptive devices: MR imaging. *Radiology*. 1987; 162:311–4. [PubMed: 3797642]
17. Moschos E, Twickler DM. Intrauterine devices in early pregnancy: findings on ultrasound and clinical outcomes. *Am J Obstet Gynecol*. 2011; 204:427.e1–6. [PubMed: 21345405]
18. Ber A, Seidman DS. Management of the malpositioned levonorgestrel-releasing intrauterine system. *Contraception*. 2012; 85:369–73. [PubMed: 22036043]
19. Prager S, Darney PD. The levonorgestrel intrauterine system in nulliparous women. *Contraception*. 2007; 75(6 Suppl):S12–5. [PubMed: 17531602]
20. Sivin I, et al. Prolonged intrauterine contraception: a seven-year randomized study of the levonorgestrel 20 mcg/day (LNg 20) and the Copper T380 Ag IUDS. *Contraception*. 1991; 44(5): 473–80. [PubMed: 1797462]
21. Bell JD, et al. The effects of a single cervical inoculation of *Chlamydia trachomatis* on the female reproductive tract of the baboon (*Papio anubis*). *The Journal of infectious diseases*. 2011; 204(9): 1305–12. [PubMed: 21921205]

Fig. 1. Magnetic resonance image of ex vivo baboon uterus with a levonorgestrel intrauterine device in place. The device appears as a band of low signal intensity (arrow) oriented parallel to the longitudinal axis of the uterus. Note that the arms of the device are only partially deployed.

Fig. 2. Representative MR image of human uterus with a levonorgestrel intrauterine device in place. The device appears as a band of low signal intensity (arrow) oriented parallel to the long axis of the uterus. Note that the arms of the device are fully deployed.