When the button is depressed, the latch releases the sliding [redacted] The compressed energy in the spring is released, however the motion of the spring is damped by the fluid filled dampener. As the spring expands, the sliding hub is retracted within the chassis, thereby pulling the electrode sheath over the Matrix (which is held in position by the fixed push rod) and releasing the Matrix from the hole in the end of the electrode sheath. (Note that the Matrix is not 'ejected forward', through the exit hole, but is rather 'uncovered' by the retraction of the sheath over the Matrix as it is held in position by the push rod.)

A more detailed explanation of the mechanical action of the catheter is contained in Section 2.4.3.

2.3.1.3 Sterilization

The Adiana Delivery Catheters are supplied sterile and will remain so as long as the unit package is unopened and undamaged. The Delivery Catheters are moist heat (steam) sterilized and are labeled for single use only. Details on sterilization processes, validation and shelf life testing will be included in Module 2.

2.3.1.4 Packaging

Each delivery Catheter is placed in a thermoformed [redacted] tray and sealed with a [redacted] lid. One end of the tray has a pre-formed Chevron seal for ease of opening.

The sealed delivery catheter in sterile barrier tray is placed in a chipboard box for protection during handling and shipping.

Primary product labels are placed on both the delivery device's tray cover and on the exterior of the chipboard box.

Additional information on product labeling will be provided in Module 4 and on product packaging in Module 2.

III. B. 2. ADIANA RF GENERATOR

2.3.2 Adiana RF Generator

2.3.2.2 Overview

The Adiana Radio Frequency (RF) Generator is intended for use with the Adiana Delivery Catheter to perform female sterilization. The Adiana RF Generator is a microprocessor-controlled, bipolar electrosurgical generator with automatic temperature control and a unique tissue contact sensor. The Adiana RF Generator uses a menu driven display to guide the operator successfully through the Adiana procedure. During use, the RF Generator constantly monitors catheter outputs and signals to determine proper device placement, to control lesion creation, to ensure Matrix delivery, and to detect error conditions. There are no user-selectable RF controls for RF output, treatment time or treatment temperature. Using less than 3 Watts of power, RF settings are pre-programmed in the software to reach and maintain a pre-set temperature for a pre-determined treatment duration (as monitored by two thermocouples positioned at the surface of the catheter). If necessary, the clinician can terminate treatment, however no other physician control of output power is provided.

2.3.2.2 General Description

2.3.2.2.1 Physical Description

Although schematics, circuit design data, and component specs will be provided in Module 2, this section will provide an overview of the Adiana RF Generator (RFG). Approximately 14"W x 18"D by 4.25"H (356mm x 457mm x 108mm), the RF Generator weighs approximately 15 pounds (6.8 kg). It has a custom sheet metal housing with a sculpted [redacted] front bezel. The front bezel houses a monochrome dot matrix transmissive LCD display with 240 x 64 pixel resolution and a viewing area of 134mm x 40.4mm.

2.3.2.2.2 Electrical Description

The RF Generator can be configured to operate at either 110V or 220V, (Part Numbers [redacted], respectively) based on regional

requirements. The RF Generator is able to output a maximum power of 5 Watts at a frequency of 460±10KHz in a sine waveform. While the generator is electrically capable of delivering up to 5 Watts of power, it is software limited to deliver a maximum of 3 Watts during treatment. Figure 2.18 below shows the Maximum Power Output versus Load for the Adiana RF Generator.

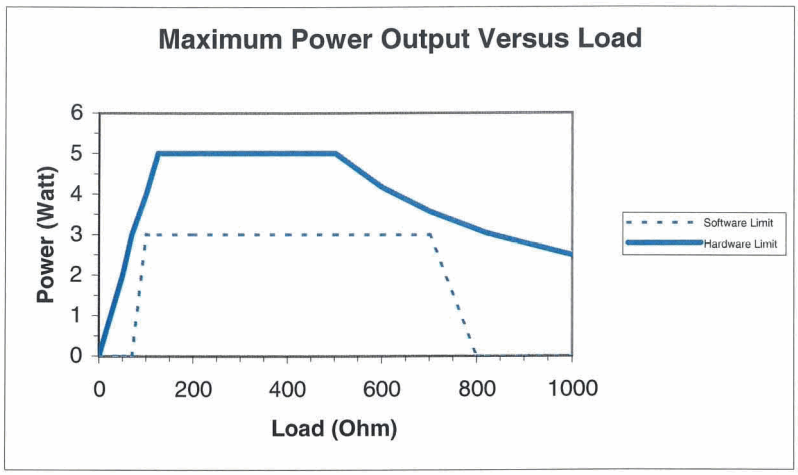


Figure 2.18: Adiana RF Generator maximum Power output versus Load Curve

2.3.2.2.3 Description of User Interface Features

Front Panel

The front panel of the Adiana RF Generator contains the majority of user controls (Figure 2.19). Buttons controlling primary functions, as well as the LCD display, and a receptacle for the cable that connects to the Adiana Delivery Catheter all reside on the front panel of the RF Generator.

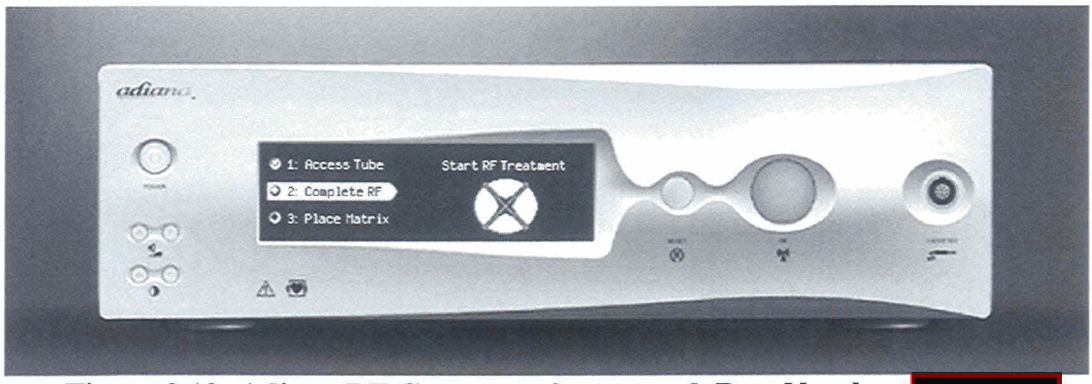
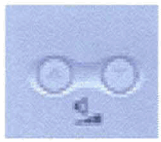
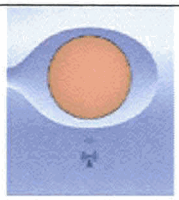


Figure 2.19: Adiana RF Generator front panel, Part Numbers [redacted] and [redacted] (Note [redacted] is a 240V input voltage configuration, which is factory selectable on the power supply. This will be described in further detail in Module 2.)

A description of the control buttons and their functions are given below. Please refer to Figure 2.19 for location of each item on the front panel.

Table 2.1: RFG Controls

Graphic Example	Description
	Power Switch -- This switch controls both the ON and OFF power. When lit, it indicates that the Power is ON.
	**Volume** **Down** – Decreases the volume of the audio speaker. A single depression of the key decreases the value by one unit. Continuous depression decrements the volume to the minimum allowable value. **Up** – Increases the volume of the audio speaker by one unit. Continuous depression increments the volume to the maximum allowable value.

Graphic Example	Description
	Contrast Changes the visual contrast of the display screen. By changing the visual contrast, the user can optimize the display appearance to compensate for the angle at which they are viewing the display. **Down** – A single depression of the key decreases the visual contrast by one unit. Continuous depression decrements the display to the lowest visual contrast. **Up** – A single depression of the key increases the visual contrast by one unit. Continuous depression increments the display to the highest visual contrast.
	RESET Stops the output of Radiofrequency energy during On Mode. Also Resets the RFG after an Error occurs. The switch is shown here lit RED which indicates that the RF Generator has entered an error mode and may be reset by pressing this button.
	**RF** - Starts and stops the output of Radiofrequency energy. The switch is unlit when RF energy is NOT being delivered. (Shown with RF Power OFF.)

Display

	Display -- In RF ON mode, displays the total remaining treatment time.

Front Panel Receptacle

	Catheter – Used to connect the proximal end of the Catheter Cable to the RF Generator. This is a 14-pin connector, designed ONLY for use with the Adiana Catheter Cable.

Adiana RF Generator Rear Panel

The rear panel of the Generator is illustrated below in Figure 2.20. The rear panel carries relevant warnings and electrical safety information as well as functional elements that are shown in Figure 2.20 and listed in Table 2.2 below:

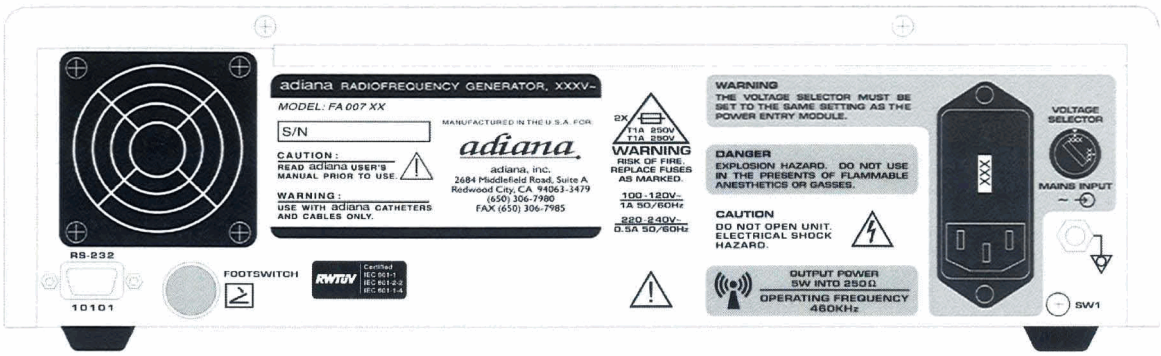
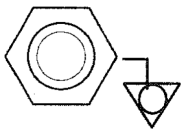
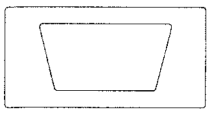
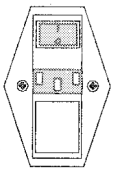
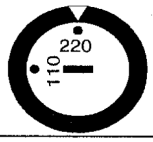
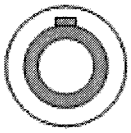


Figure 2.20 Adiana RF Generator Rear Panel [redacted] (110VAC) and [redacted] (220VAC)

Table 2.2: Rear Panel Functional Elements

Rear Panel	Description
	**Equipotential Ground Stud** -- Provides a means of securely linking the earth grounds of the Generator to other grounded equipment.
RS232  10101	**RS-232 Port** – For Service Use Only.
	**AC power inlet** -- IEC inlet power entry module with fuses, EMI filter, and AC switch.
VOLTAGE SELECTOR 	**Voltage Selector** – SERVICE ONLY: This switch configures the Generator to either 110V~ or 220V~, in conjunction with internal setting of the Power Entry Module. The configuration may only be changed by qualified service personnel with the appropriate tools.
	**Footswitch Receptacle –** Used to connect Adiana Footswitch to the Adiana RF Generator.

2.3.2.3 RF Generator Accessories

2.3.2.3.1 Optional Footswitch

The optional Adiana Footswitch allows hands free operation of the Adiana RF Generator. The footswitch is equivalent to the RF button on the Generator's front panel. Connected to the back of the generator, the footswitch is pneumatic and has a guard housing. Used solely as an alternative mechanism for engaging the RF energy emission operation, the footswitch operates an internal switch within the RF Generator.

During the course of updating the IEC certifications, it was discovered that the circuitry for the original footswitch used in the RF Generator was not IEC 601 compliant. A redesign was undertaken to utilize a compliant circuit; a pneumatic footswitch which actuated an internal switch was selected to avoid a non-isolated circuit outside the RF generator enclosure. The following changes were made to the generator to achieve compliance:

- Change to a pneumatic footswitch
- Addition of a pneumatic module inside the RF Generator
- Changing from an electrical connector to a pneumatic connector
- Changes to the Operating System software to sense this signal

Changes to the RF Generator hardware and software specifications and validation of the changes will be provided in Module 2.

2.3.2.3.2. Adiana RF Generator Cable

The RF Generator Cable is a custom cable assembly used to connect the Adiana Delivery Catheter to the front receptacle labeled "CATHETER" on the Adiana RF Generator. The Catheter Cable is supplied non-sterile.

2.3.2.4 Electrical Certification and Packaging Validation

The Adiana RF Generator originally received its RWTUV Mark Approval Certificate on May 30th, 2001. This mark certified compliance with IEC 601-1-1, IEC 601-2-2, and IEC 601-1-4. In addition, Advance Compliance Engineering Services certified compliance with IEC 601-1-2. Following minor changes to the RF Generator, the Adiana RF Generator was re-certified by RWTUV on April 7, 2006. (See Section 4.2.4.)

The RF Generator was tested and passed Packaging and Thermal Qualification tests performed by [redacted] Tests included those outlined by ASTM

D4169-99. Packaging verification testing will be discussed in Module 2.

2.3.2.5 RF Generator Functional Description

There are five functional elements within the RFG. They are the Power Supply, CPU board, Radiofrequency (RF) board, front panel board, and the [redacted] board (Figure 2.21). The CPU board controls RF delivery, monitors front panel switches, and controls all displays. The RF board delivers radiofrequency energy as well as provides measurements of voltage, current, power, and individual return currents. Front panel boards act as an interface between the CPU and the graphic display and the front panel switches as well as providing the circuits for controlling loudspeaker volume and display contrast. The measurement board or [redacted] board provides electrical isolation and signal conditioning for the thermocouples, the Position Detector Array (for monitoring tissue contact), and matrix ejection sensor.

Figure 2.21: Functional Block Diagram: Adiana RF Generator

The power supply used within the RF Generator to provide power to system circuitry is isolated from the lines (mains) by a commercially available, medical power supply, and by transformer and optical isolation on the RF board.

III. C. PRINCIPLES OF OPERATION

2.4 Principles of Operation

2.4.1 Device Action

Use of the Adiana Transcervical Sterilization System is based on a simple two step process:

1) Access the fallopian tube and create a superficial tissue injury using controlled delivery of RF energy into the tissue, and

2) Place a porous biomaterial implant into the fallopian tube at the site of the thermal lesion, leading to tissue in-growth and tubal occlusion

The Adiana System is intended to be used with standard hysteroscopic techniques and equipment to allow for transcervical access to the fallopian tube.

The following section describes in detail the actions of both the Adiana RF Generator and the Adiana Delivery Catheter to accomplish this process.

2.4.2 Typical Treatment Sequence

The Adiana RF Generator utilizes a menu driven display to guide the user through the various steps of the Adiana procedure. As the Generator transitions from one mode to the next it continuously checks for errors in the catheter, as well as within the generator itself. For *reference only*, a copy of the current Adiana RF Generator Users Manual is included in Appendix 34. Complete labeling will be provided in Module 4.

Details of the mechanical action of the Adiana Delivery catheter are included in Section 2.43, following this section.

<u>The RF Generator Treatment Sequence is described below:</u>

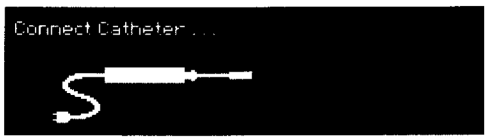
<u>Power On Self Test (POST)</u>

When main power switch is turned on, the system performs internal self tests (or Power On Self Tests (POST)). If the test fails, then the unit will go to the FAULT Mode with the appropriate diagnostic

message. If the test passes, the system enters the *Connect Catheter* mode.

Connect Catheter

This mode is entered automatically after a successful internal self-test. It will also be entered from either the *Access Tube* or *Complete RF* modes if the catheter is disconnected.



After a catheter is connected it is immediately tested for functionality (for example the thermocouples are tested for continuity, and all electrical lines are checked for shorts). If any error is found the appropriate error message is displayed. If the catheter passes these checks, then the Generator transitions to *Access Tube* mode.

Access Tube

During this mode the Generator is continuously monitoring the four Position Detector Array (PDA) channels on the Delivery Catheter for tissue contact. As the catheter is advanced into the fallopian tube, each channel comes into contact with the fallopian tube wall and the corresponding quadrant in the PDA display is illuminated. Only after all four PDA channels measure predetermined impedance set points will all four quadrants be illuminated.

At this point the user has successfully placed the Delivery Catheter within tubal ostium and a blinking RF button prompts the user to begin RF treatment. If any electrical failure is detected during *Access Tube* mode the Generator displays the appropriate error message. After the user presses either the RF Start/Stop button on the front panel or depresses the foot pedal, the Generator transitions to *Complete RF* mode.

Complete RF

During *Complete RF* mode the RF Generator is delivering RF energy through the catheter. The duration of RF Energy delivery is 60 seconds. The LCD display transitions to a screen with a countdown timer, and an audible tone is generated at 5 second intervals over the course of the treatment.

Over the course of energy delivery, various aspects of the device are monitored to ensure that the RF treatment is successful: the thermocouples are continuously monitored; all current carrying elements are monitored for shorts and open circuits and the PDA is continuously monitored to ensure that the Delivery Catheter is still within the fallopian tube during treatment. Energy to the Delivery Catheter is automatically controlled via feedback from the control thermocouple.

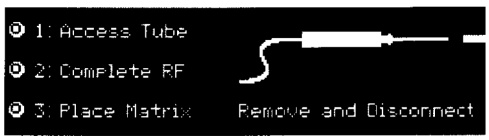
[redacted]

During the *Complete RF* mode a second diagnostic thermocouple is continuously monitored to ensure that heating is occurring evenly over the length of the catheter's electrode array. After the 60 second RF treatment is successfully completed, the Generator transitions to *Place Matrix* mode.

Place Matrix

This mode is entered following the *Complete RF* Mode after normal completion of the RF delivery. The operator is alerted to the end of the RF with an audible tone and the visual display as shown below:

The operator then releases the matrix by depressing the push button on the Adiana catheter handle. A compressed spring within the handle is released, retracting the sheath and exposing the Matrix. When the operator releases the Matrix, the system will sense the matrix ejection, and then will transition to the *Connect Catheter* Mode, notifying the operator to remove and disconnect the Adiana delivery catheter, as seen below:



If the Delivery Catheter is disconnected before the Matrix is ejected, an error is triggered.

2.4.3 Catheter Mechanical Action: Matrix Placement Sequence:

After completion of RF power delivery, the RF generator notifies the clinician to place the matrix. The matrix release mechanism consists of a series of mechanical connections between the push button and the electrode sheath:

> The push button, when depressed, releases an internal latch. Energy, stored in a simple spring within the spring dampener, is released at a controlled speed, retracting a sliding hub. The continuous electrode sheath extends all the way back to the handle. The sheath is attached to the sliding hub.
>
> The push rod assembly forms a rigid, stationary assembly extending from it's fixed end, which is attached to the handle chassis, to it's distal end, which rests against the proximal end of the matrix.
>
> When the sliding hub is retracted into the chassis, the sheath is retracted relative to the stationary push rod, thereby releasing the matrix. Indeed, the matrix is not 'ejected through' the exit hole, but is rather 'uncovered' by the retraction of the sheath over the matrix as it is held in position by the push rod.

The following sequence of photographs detail this process. (refer to Figures 2.22 through 2.25).

[redacted]

Figure 2.22: Schematic Drawing of the current handle in 'as shipped' configuration. [The outer handle covers, wires, wire connectors and extraneous parts are omitted for clarity.] Note that the spring is fully compressed, and the sliding [redacted] is fully forward. The latch is holding the sliding [redacted] in this position. The outer sheath, which extends continuously to the distal end of the catheter to form the electrode sheath, is attached to the Sliding [redacted]. The [redacted] forms the proximal end of the pushrod assembly, which is likewise continuous to the distal end of the catheter. The outer catheter shaft is not shown over the sheath and pushrod for clarity.

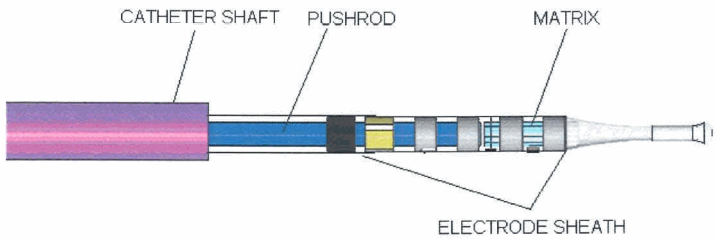


Figure 2.23: Distal tip detail. Note that the push rod assembly is directly behind the Matrix, which is contained under the electrode sheath.

[redacted]

Figure 2.24: This drawing shows the device in the fully released position. The spring has fully extended, retracting the sliding [redacted] back to the distal most position in the [redacted] (Note that once the sliding [redacted] has moved a distance sufficient to release the Matrix, a sliding contact within the handle (not shown) is opened, signaling Matrix release to the Adiana RF Generator. The RF Generator then sounds an audible tone and displays an indicator signaling Matrix release.)

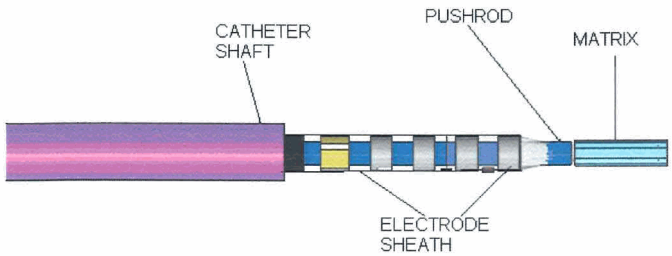


Figure 2.25: Distal end of the catheter showing Matrix release. The sheath has been retracted over the push rod into the end of the catheter shaft, exposing the Matrix which 'exits' the catheter tip through the Matrix exit [redacted]

III. D. CLINICAL SEQUENCE: ADIANA PROCEDURE

2.5 Clinical Sequence: Adiana Procedure

The following sections, 2.5.1 through 2.5.4, contain a summary of the detailed clinical procedures utilized within the EASE clinical trial for patient preparation, treatment and discharge. These steps have been excerpted from the protocol and are as follows:

2.5.1 Preparation of Equipment

- Equipment should be set up prior to the start of the procedure.
- Adiana RF Generator is plugged into the wall power receptacle.
- Adiana RF Generator is energized by pressing the green power switch.
- Adjust display contrast and volume tones as required.
- Verify successful completion of the power-on self diagnostics (display should indicate "connect catheter").
- The Adiana Generator Cable should be connected to the RF generator

2.5.2 Patient Preparation

- The patient should be prepped and draped per local practice.
- The equipment for the Adiana System should be connected and positioned for convenient physician operation.
- A pulse oximeter should be used to monitor the patient's pulse and oxygen saturation. (NOTE: Respiration, pulse, and oxygen saturation should be monitored carefully throughout the procedure.)
- Administration of local anesthesia, oral analgesia/sedation, and/or conscious (IV) sedation to prevent discomfort is at the discretion of the physician. (IV sedation should be used only when necessary, and general anesthesia is discouraged.) . However, if necessary for patient comfort, IV sedation may be considered. Paracervical block may be administered by using Lidocaine or equivalent injections into the cervix in the standard fashion (NOTE: Adequate time for the local anesthetic to take effect should be allowed).

2.5.3 Patient Treatment

Some or all of the following steps may be performed immediately prior to the start of the hysteroscopic procedure:

- Prior to the use of the Adiana Delivery Catheter, the device should be carefully inspected through the sterile barrier tray to determine if there has been any damage. If there has been any damage to the device or its packaging, the device should not be used.
- Using standard sterile technique, the Adiana Delivery Catheter and Adiana Split Introducer should be removed from the sterile barrier tray.

- The distal-tip protective cover should be removed from the end of the Adiana Delivery Catheter.
- The Adiana Delivery Catheter should be connected to the Adiana RF Cable. The generator display should indicate "Access Tube".

2.5.3.1 Hysteroscopic Access:

- The vagina and vulva should be cleaned with an antiseptic solution.
- A speculum should be inserted into the vagina and the cervix exposed.
- The sheathed hysteroscope with fiber optic light source should be inserted transcervically to the cornual region of the uterine cavity. (Distension media should be flowing prior to hysteroscope insertion.) Prior to hysteroscope insertion, a cervical dilator may be used as required at the discretion of the physician.
- A nonconductive distention medium (Glycine) should be applied via the hysteroscope. Applied pressure should be limited to less than 150 mm Hg. CAUTION: Duration of procedure and total volume of fluid applied should be monitored to ensure that fluid overload does not occur. Inflow and outflow should be monitored by medical staff. If at any time the fluid deficit exceeds 800cc, the procedure should be terminated.
- The uterine cavity and tubal ostia should be examined. If excluding factors are found, e.g. presence of lesions preventing access to the intramural portion of the fallopian tube, the Adiana Procedure should not be performed.

2.5.3.2 Device Placement

- Both tubal ostia should be visually located prior to placement of the Delivery Catheter into the hysteroscope.
- Once the Delivery Catheter is introduced, using the Adiana Split Introducer, the first tubal ostia should be visualized and cannulated; once the RF generator indicates tubal contact, RF energy is applied.
- Treatment is automatically controlled at 64°C for 60 seconds.
- The electrode sheath should then be retracted, exposing and placing the Matrix.
- After successful completion of the first tube, the contralateral ostium should then be treated with a new Delivery Catheter, including RF and matrix placement.
- If either the first or second access is unsuccessful, the procedure should be terminated

Figure 2.26 shows a pictorial view of the Adiana procedure on a single fallopian tube.

2.5.4. Post-Treatment

If the patient has received IV sedation, she should be monitored for no less than 2 hours post procedure. Prior to release, each patient should:

- Have TVUS imaging performed prior to discharge to monitor the position of the matrix. (TVUS imaging is nonetheless recommended at the One-Week follow up visit.)
- Be given a prescription for alternative contraceptives (either Oral Contraceptives (OC's), cyclic contraceptive preparation (CCP's), or condoms or diaphragm (with spermicide) and explicit instructions.

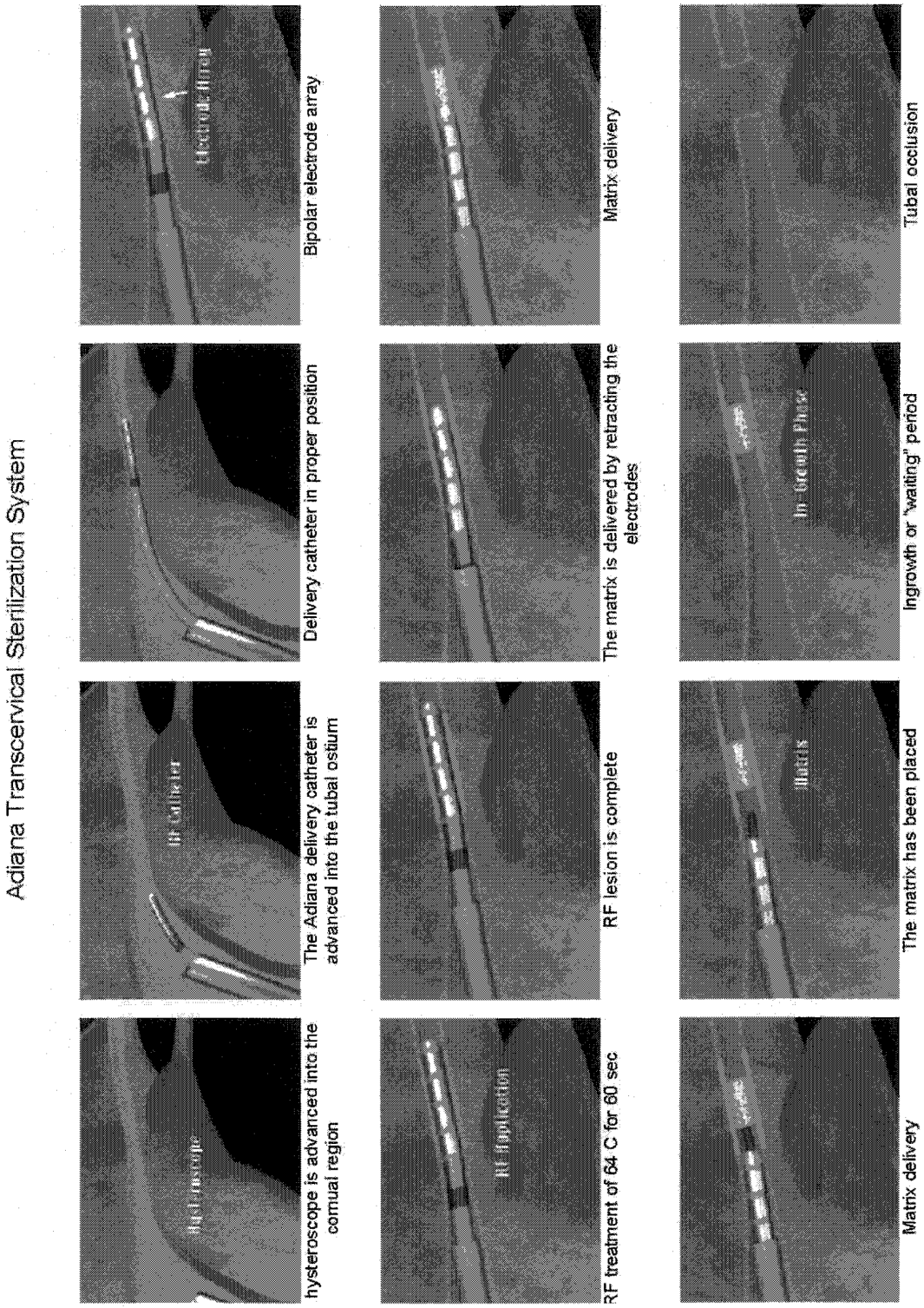


Figure 2.26: Simulation showing the steps of the Adiana TranscervicalSterilization Procedure.

III. E. MECHANISM

4.3.1. Surgical Procedure and Deployment

The Adiana treatment involves: 1) hysteroscopic, transcervical introduction of a catheter, 2) hysteroscopically controlled insertion of the distal tip of the catheter device into the fallopian tube lumen, 3) bipolar radiofrequency current application to the tubal lumen, and 4) implantation of a biomaterial matrix or tissue scaffold in the region of the radiofrequency lesion within the fallopian tube, and 5) ingrowth of healthy tissue into the implant, and incorporation of the implant, to create a long-term blockage.

The underlying mechanism proposed by Adiana consists of the following components: First, the delivery of radiofrequency current destroys the epithelial lining of the fallopian tube lumen, and stimulates a wound healing response. Then the matrix is delivered into the RF treated segment of the fallopian tube. The matrix has been designed to provide a suitable architecture for tissue in-growth during this wound healing response. Finally, the wound healing response into this unique tissue scaffold results in a complete integration of healthy tissue into the implant. The action of the matrix is not simply to mechanically occlude the tube, but rather to provide a tissue scaffold leading to permanent occlusion through the natural healing response.

IV. PRODUCT DEVELOPMENT

IV. A. BENCH

5. Executive Summary: Testing Contained in Module #1

- Material Safety Testing

Material Safety Testing of the Adiana Delivery Device and Matrix was performed to ensure that raw materials, manufacturing, and sterilization processes have resulted in a biocompatible product. The Adiana Delivery Device is considered a "Surface Contact" device that contacts breached or compromised surfaces (Adiana considers the interior of the fallopian tube "compromised" following lesion formation) with contact duration less than 24 hours. This testing also meets testing guidelines for limited duration contact with mucosal surfaces. The Matrix, delivered by the delivery device, is an "Implant Device", which contacts tissue, with permanent contact duration.

The implanted component, the Adiana matrix, comprised of [redacted] has been tested separately and together with the Delivery Catheter. Additionally, non-carcinogenicity of this material has also been established through [redacted] testing, Adiana bench and animal testing, historical data, and information provided by the manufacturer. Results are further presented in Sections 4.1.2 (Material Safety), Section 4.2 (Engineering/Bench Testing) and Section 4.3 (In Vitro and Animal In Vivo Studies).

Patient contact materials used in various components of the Adiana Transcervical Sterilization System are listed in Table 4.2.

All product biocompatibility testing was performed following steam sterilization. The majority of the testing was conducted by [redacted]

Using its In-growth Scoring System, Adiana has also conducted a series of safety/performance animal studies to evaluate long term safety and efficacy. These studies are described further in Section 4.3. Overall, these studies showed no unusual histological or carcinogenetic implant effects.

After exposure to its steam sterilization cycle, Adiana's Transcervical Sterilization System meets the biocompatibility requirements for cytotoxicity, genotoxicity, irritation, acute systemic toxicity, and sensitization. Results are presented in Section 4.1.2 Material Safety. It has been shown to be fully compliant with the biocompatibility requirements of ISO 10993-1 and is determined to be acceptable.

- Engineering/Bench Testing

Engineering / Bench testing of the Adiana Transcervical Sterilization System has been conducted in order to verify that the design output conforms to the design input requirements described in the product specification. Description and results of bench testing are reported in full in Section 4.2 for the Adiana Delivery Catheter and Matrix (for both the "Current" and "Original" handles). Per agreement with FDA, testing for the RF Generator will be reported in Module #2.

While Material Biocompatibility is addressed in Section 4.1 of this Module, additional information describing patient contact materials is provided in the Material Analysis Section 4.2.2. of the Engineering/Bench Testing section. Product specifications and inspection criteria are described for each patient contact material including Silicone [redacted] Stainless Steel, [redacted] Tubing, Extrusion [redacted] Composite [redacted] tubing with [redacted] .056"ID, .002 wall, black; and Lubricious Coating.

Verification test results for the Current Delivery Device [redacted] that were submitted as [redacted] and approved [redacted] are presented in Table 1.2 below and are described more fully in Section 4.2.3.1 (Engineering/Bench Testing).

[redacted]

Test	Units Tested	Results:
[redacted]		

All results obtained during this evaluation were acceptable. The results verify that the design output conforms to the design input requirements described in the product specification.

Additionally, two minor interrelated post-trial device modifications to the "Current" Delivery Catheter are described in Sections 2.6.2 and 4.2.3.2. These changes were developed in response to device malfunctions in the field. The band spacing modification and change in shape to the tip of the push rod are both within original product specifications.

Section 4.2.3 of Engineering/Bench Testing also contains description and verification testing for the "Original" Delivery Device and Matrix (Section 4.2.3.4). A description of the Compression Testing that was performed separately on the Matrix as part of the original Design Verification testing is included (Section 4.2.3.5). Finally, a summary of the [redacted], submission of minor changes to the "Original" Delivery Catheter [redacted] is provided (Section 4.2.3.2.).

- Electrical Safety Testing

The Adiana RF Generator was designed and developed by [redacted] under contract to Adiana. All product design activities were performed in accordance [redacted] written product development procedures. [redacted] is in compliance with ISO 13485. In addition, [redacted] will manufacture the Adiana RF Generator. Module 2 will contain a complete discussion of the [redacted] manufacturing facilities, Quality Systems procedures, and Design Control systems. Module 2 will also include a detailed product description and testing verification results for the RF Generator hardware and software.

Electrical Safety and electromagnetic compatibility testing of the Adiana RF Generator was performed on both the Original and Current handle devices. Testing was conducted in accordance with internationally recognized standards by [redacted] and by independent test facilities. Certification of Compliance and/or documentation of successful test results for the Adiana RF Generator are summarized in Table 1.3 below.

[redacted]

TEST	RESULTS
[redacted]	

- Development Studies

  Adiana has performed an extensive series of investigations to characterize the safety of the Adiana procedure, to determine the proper device configuration and to define a clinical protocol. This research has focused on all aspects of device performance, including access to the fallopian tube, radiofrequency lesion creation, matrix delivery, host tissue response and in-growth, tubal occlusion and pregnancy prevention. These studies, which are presented in detail in Section 4.3 (In Vitro and Animal In Vivo Studies) are defined below (note that additional human clinical development studies will be presented in Module 4):

  In Vitro Extirpated Uteri Studies:

  These studies utilized extirpated uteri from patients undergoing elective hysterectomy. Following removal, the uteri were used to evaluate catheter handling and performance. In addition, the ability to deliver RF energy and create a superficial thermal

destruction of endosalpynx to the epithelial layer in the fallopian tube was evaluated. Histological analysis following treatment in extirpated uteri samples was used to define proper RF treatment parameters.

Animal Studies:

The Adiana system was evaluated in the rabbit model. This model was used primarily to assess: acute RF performance, tissue in-growth into the matrix, fallopian tube blockage and pregnancy prevention. Animal studies have confirmed that the Adiana system results in tubal occlusion as demonstrated by rigorous challenge with pressurized fluid. In addition, mating studies performed in rabbits have shown pregnancy prevention at 6 months and 12 months post implant.

IV. B. BIOCOMPATIBILITY

4.1.2. MATERIAL SAFETY

Material Safety testing of the Adiana Delivery Catheter and Matrix were performed to ensure that raw materials, manufacturing processes and sterilization processes result in a biocompatible product. Material Safety testing of the Adiana Delivery Catheter and Matrix was performed with written protocols, in accordance with ISO 10993, International Standard for Biological Evaluation of Medical Devices, Part 1: Guidance on Selection of Tests; Part 2: Animal Welfare Requirements; U.S. Office of Device Evaluation General Program Memorandum #G95-1, Use of International Standard ISO-10993: Biological Evaluation of Medical Devices Part 1: Evaluation and Testing, May 1, 1995.

Adiana's Material Safety testing was conducted according to protocol and reported in [redacted] These reports describe Adiana's biocompatibility testing and the rationale for those tests. Results of this testing are summarized in Tables 4.3 and 4.4. Detailed reports can be found in Appendices 7-10.

Since the Adiana RF Generator and accessories are not in patient contact, only the Adiana Delivery Catheter with the implantable Matrix is subject to biocompatibility consideration.

The implanted component, the Adiana Matrix, comprised solely of [redacted] material, is purchased in its raw uncured form from [redacted] and converted to a fully cured material by way of Adiana's manufacturing process.

Non-carcinogenicity of the matrix silicone implant material has been demonstrated by several means that are listed here and discussed more fully in Section 4.1.2.6 below:

1) Biocompatibility Testing
2) Pre-clinical animal studies
3) Early stage clinical studies
4) [redacted] testing
5) Historical data

4.1.2.1. Reference Documents

[redacted]

21 CFR Part 58 – Food and Drug Administration, Good Laboratory Practice for NonClinical Laboratory Studies, September 4, 1987

ISO 10993-1: 1997. Biological evaluation of medical devices – Part 1: Evaluation and Testing

ISO 10993-2; 1992. Biological evaluation of medical devices - Part 2: Animal Welfare Requirements

General Program Memorandum #G95-1, Use of International Standard ISO-10993, Biological Evaluation of Medical Devices Part 1: Evaluation and Testing, U.S. Office of Device Evaluation (ODE) May 1, 1995

MAF # 870, Device Master File for MED-4860 silicone elastomer, filed with U.S. Food and Drug Administration. (See Appendix 5 for NuSil's Permission to Reference Letter)

PS 008: Adiana System Delivery Device Product Specification. ("Original" catheter design)

PS 0011: Adiana System Delivery Device Product Specification ("Current" catheter design)

Scientific Justification for the Deletion of Certain Biological Tests from the Testing Scheme Proposed in the FDA's "Guidance for Manufacturers of Silicone Devices Affected by the Withdrawal of Dow Corning Silastic Materials" , Schulz, Carl, Ph.D. COSAR Inc., July 26, 1993. Prepared for NuSil Technology, Carpinteria, CA (Attachment 9.16)

4.1.2.2. Patient Contact Materials

The Device Delivery Catheter is a "surface contact" device that contacts breached or compromised surfaces (Adiana considers the interior of the fallopian tube "compromised" following lesion formation) with contact duration of less than 24 hours. Biocompatibility testing also meets guidelines for limited duration contact with mucosal surfaces.

The Matrix delivered by the Delivery Catheter is an "Implant Device" which contacts tissue, with permanent contact duration. The Matrix is manufactured from fully cured silicone elastomer. The raw material is purchased from [redacted] Although Adiana utilizes different curing parameters than [redacted], Adiana processing methods (including curing and steam sterilization) do not introduce any additional compounds into the final implant material. Therefore, the manufacturer's test results for the supplied material are relevant and included in this report. Adiana has also performed similar testing on the [redacted] raw material cured with Adiana manufacturing process. Additional information about the biocompatibility of this material can be found in Sections 4.1.2.5 and 4.1.2.6 of this module.

Table 4.2 designates patient contact materials from the "Original IDE" device, those that were used in the interim device that was approved by [redacted] While many materials remain unchanged, some materials were eliminated or introduced based on new structural changes.

Both the original and current Delivery Devices contained matrices at the time of testing and have been fully biocompatibility tested per the Limited Duration Tissue Contact testing protocol testing methodology per ISO 10993-1. This testing included Cytotoxicity, Sensitization, Irritation, and Acute Systemic Toxicity. Testing on the Matrix was not repeated as there were no changes to the matrix composition or to the materials in contact with the Matrix. Biocompatibility results for the original Delivery Device and Matrix are found in Table 4.3 while Biocompatibility results for the current Delivery Device and Matrix are found in Table 4.4. Detailed reports of all testing can be found in Appendices 7 through 10.

[redacted]

[redacted]

[redacted]

[redacted]

4.1.2.4. Adiana Transcervical Delivery System Material Safety Summary

Summary of Material Safety Test Methods

- Cytotoxicity Test using the ISO elution was performed to detect potential cellular abnormalities in the L-929 Mouse Fibroblast Cell Line.

- Delayed Contact Sensitization Study (Maximization Method) using the guinea pig, was performed to evaluate the potential for a dermal contact sensitization.

- Irritation (Acute Intracutaneous Reactivity) was performed to detect the presence of intracutaneous reactivity including erythema and edema following injection into rabbit skin.

- USP Systemic Toxicity was performed to determine whether leachables extracted from the test article would cause systemic toxicity in mice, post injection.

- Genotoxicity testing was completed to evaluate the potential of the test article extract to cause genotoxic changes in chromosomes or in the mitotic apparatus of murine polychromatic erythrocytes (PCEs).

- USP Muscle Implantation testing was conducted in the rabbit to determine local tissue response from test material samples with histology at 1 week.

- USP Intramuscular Implantation testing was performed in the rabbit to determine local tissue response from test material samples at 12 weeks.

- Microsome Mutagenicity Testing (Microsome Mutagenicity Assay) was conducted to determine whether a saline extract of the test article causes mutagenic changes in Salmonella typhimurium.

- Pyrogenicity Study (Material Mediated) was conducted to determine whether 0a saline extract of the test article causes pyrogenic changes in the rabbit.

- *In vitro* Hemolysis study (Extraction Method) was performed using whole rabbit blood to determine if an extract of the test article would cause hemolysis (cell lysis) *in vitro*.

- Pre-clinical testing was performed in rabbit studies. The data collected in these studies (specifically histology data) supports the in vivo safety of the matrix implant.

- Early-stage clinical testing was performed using the pre-hysterectomy model including in vivo implants for periods up to approximately 12 weeks. These evaluations were performed with the full Adiana system; therefore, the data collected supports the safety of the Adiana system and its individual components, i.e. RF Generator, delivery catheter, and matrix. The evaluations performed in these studies included safety endpoints, supported the in vivo safety of the Matrix implant through histology data.

Tables 4.3 and 4.4 below summarize biocompatibility test results for both the Original Delivery Device and Matrix, and Current Delivery Device, respectively.

[redacted]

[redacted]

[redacted]

4.1.2.5. Histology Data Supporting Implant Tolerance

Pre-clinical testing was performed in rabbit studies. The Matrix was implanted long-term (for periods up to 12 months) in rabbit fallopian tubes. These studies varied in their purposes and endpoints; however, all studies ended with animal sacrifice, explant of the Matrix and surrounding tissue, and detailed histological analysis by qualified microbiologists. Adiana Protocol [redacted] (Appendix 28) specifically included a toxicology analysis of rabbits implanted for 9 months, and demonstrated blood and cell counts that had no unusual or unexpected alterations at the completion of the implant period. The data collected in these studies (especially histology data) support the safety of the Matrix by demonstrating that the surrounding tissue tolerates the Matrix implant. Specifically, multiple parameters were used in assessing the histological in-growth and deriving the biomaterial response with the host. Quantitative assessments were used for measurements of closed vascular structures or blood vessels, percentage of remaining epithelium and inflammatory cells, and qualitative assessments were used for the observations of giant cells, necrosis and fibrotic capsule. No significant adverse findings were reported from gross histologic observations. A table below provides summary information on these rabbit studies:

Table 4.5: Rabbit Study Summary Information

Study Title	Adiana Report	Number of Matrices Implanted	Implant Period	Conclusions
9 Month Long-Term Implant	[redacted]	6	9 months	No adverse safety observations in histology analysis
Long Term Implant Sterilization Method Evaluation		6	6 months	No adverse safety observations in histology analysis
		6	12 months	
Long Term Implant II Matrix Length Evaluation		14	6 months	No adverse safety observations in histology analysis
		16	12 months	

During the course of several pilot studies and one pivotal pre-hysterectomy study, Adiana collected human histology data. The studies included evaluation of in vivo implants for periods up to 12 weeks. These evaluations were performed with the full Adiana System; therefore, the data collected supports the safety of the Adiana System and its individual components, i.e. RF Generator, delivery catheter, and matrix. These clinical studies:
1) evaluated the Adiana treatment against safety endpoints, 2) supported the in vivo safety of the matrix implant through histology data, and 3) noted the absence of any adverse clinical events. At the conclusion of each study, matrices and surrounding tissue were explanted and processed for histological analysis. The histological analysis of the biomaterial response with the host

was measured by quantitative counts of closed vascular spaces, residual epithelial cells, and inflammatory cells and graded assessments of giant cells, fibrotic capsule formation and necrosis. In summary, no significant adverse findings were reported from histologic observations. Discussion of these studies will be included in PMA Module #4.

4.1.2.6 Additional Matrix Non-Carcinogenicity Testing Summary

The Adiana Matrix is made from polydimethylsiloxane, a silicone elastomer that is generally regarded as safe. It is supplied by [redacted] This material is fully cured into a solid silicone form and has a long history of use in medical devices. Silicone elastomer is a flexible and stable material within the body. This material is used in a variety of prostheses including finger joints, blood vessels, heart valves, outer ears, pacemaker leads and chin and nose implants. Adiana's cylindrically shaped Matrix contains a solid core and a porous outer surface. The matrix diameter is approximately 1.60 mm and the length is approximately 3.5 mm.

As described in prior submissions, the Adiana Matrix (implant) is exceedingly small. The weight of each Matrix is approximately 3mg. If one assumes a 50kg (110lb) patient mass, with two matrices implanted, the ratio of the implant to body mass is approximately 1.2×10^{-7} or 0.00000012:1.

[redacted] is a suitable material for this gynecological application because of its inertness and mechanical properties. Non-carcinogenicity of the Matrix, comprised of [redacted] has been established through several paths including material safety testing, pre-clinical studies, [redacted] testing, and historical data. These are discussed separately below.

1) [redacted] Testing

Adiana purchases silicone from [redacted] which has a [redacted] filed with FDA. [redacted] has granted approval to Adiana to utilize this material in support of any regulatory filings [redacted] attached in Appendix 5). The [redacted] contains the results of applicable chemical and mechanical tests as well as confirmatory biological testing. Additionally, each lot of material received from the vendor contains a Standard Material Certification that includes results from mechanical testing, cytotoxicity, and trace element contamination. (Sample Certification Letter contained in Appendix 17).

In order to characterize the toxicological profile produced by treatment with silicone, [redacted] conducted silicone immunotoxicity studies in

mice at 10 and 180 days. These studies found that subcutaneous implantation of the silicone preparation produced no significant changes; there was no implant-related mortality from exposure and no gross pathologic findings at the time of necropsy. Bone marrow cellularity and CFU-M and CFU-GM stem cells were unaffected by the implants. Additionally, NuSil conducted implantation testing at 1 and 12 weeks with no toxicological findings.[1]

[redacted] is the EU Distributor of [redacted] products. This ISO9001 and ISO14001 compliant company has developed a classification system [redacted] Technology healthcare products. Materials are classified as either unrestricted (may be considered as candidate materials for any application including long term implantation of greater than 29 days) and restricted products (limited to external use or for short term implant applications of 29 days or less). They classify [redacted] as **Unrestricted** (See Appendix 14).

2) Pre-clinical Testing in Rabbits

Adiana performed pre-clinical rabbit studies. The Matrix was implanted long-term (for periods up to 12 months) in rabbit fallopian tubes. All studies ended with animal sacrifice, explant of the Matrix and surrounding tissue, and detailed histological analysis by qualified microbiologists. Adiana Protocol [redacted] specifically included a toxicology analysis of rabbits implanted for 9 months, and demonstrated normal blood and cell counts at the completion of the implant period. Additionally, no significant adverse findings were reported from gross histologic observations, e.g. no tumors were observed at the implant sites. Table 4.5 above provides summary information on these rabbit studies.

3) Early-stage Clinical Studies

The human histology data from Adiana's pilot and one pivotal pre-hysterectomy studies showed no carcinogenetic implant effect. These studies assessed tissue histology of implanted subjects at 6 weeks and at 12 weeks. No significant adverse findings were reported from gross histologic observations, e.g. no tumors were observed at the implant sites. In the tissue surrounding the Matrix, there was no evidence of cellular change or tissue change, e.g. "cellular Matrix" alteration.

[1] NuSil test 96C 10434 00, Performed at Viromed Lab: Project No 3323 (12 weeks), USP Implantation Test in the Rabbit (1 and 2 weeks) (See Appendix 65)

4) Biocompatibility Testing

As part of Adiana's Material Safety testing, the Matrix and/or Matrix material were included in and passed the following product biocompatibility tests: Cytoxicity, Irritation, Sensitization, Acute Systemic Toxicity, Pyrogenicity, Genotoxicity, Mutagenicity, and Implantation. The testing, combined with histological data from implant studies, demonstrated that the implant is benign and well tolerated by surrounding tissue. Complete specific Material Safety Test Reports referenced in this PMA module can be found in Appendices 7-10.

5) Historical data

There is ample historical data supporting non-carcinogenicity of the silicone implant. Of particular note is a white paper by Dr. Carl Schultz entitled "Scientific Justification for the Deletion of Certain Biological Tests from the Testing Scheme Proposed in FDA's Guidance for the Manufacturers of Silicone Devices Affected by the Withdrawal of Dow Corning Silastic Materials" (See Appendix 13). This document provides a justification for the use of specific biocompatibility tests for product review and approval. Additional studies are described below.

Implantable medical devices containing silicone have been widely used for a variety of medical and gynecological treatments.[2] Generally, these implantable silicones are made of closely related polysubstituted siloxanes that contain simple alkyl groups for cross-linking and specific control of physical properties. Most of the information supplied regarding local and systemic toxicity, genetic toxicity, reproductive toxicity, and carcinogenicity reports little local reaction except to smooth surfaced implants.[3] Numerous animal studies have demonstrated that the physical characteristics of the silicone implant, and not the chemical composition, are the critical determinants of carcinogenicity.[4]

[2] P920046; Filshie Clip System™; September 5, 1996 (See Appendix 11)
[3] Silicone Gel Breast Implants, The Report of the Independent Review Group, www.cosmeticsupport.com/info/silicone/toxic.htm. (See Appendix 13)
[4] James SJ, Pogribna M, Miller BJ, Bolon B, Muskhelishvili L., "Characterization of cellular response to silicone implants in rats: implications for foreign-body carcinogenesis," Biomaterials, 1997 May:18 (9):667-75. (See Appendix 13)

Summaries of Safety and Effectiveness report long term uterine carcinogenicity testing in mice and rats with no evidence of toxicological effects as a result of the silicone material.[2] The Filshie Clip System™, indicated for permanent female sterilization, utilizes a cross linked silicone elastomer rubber lining in its implantable device. This device has been on the market since 1982 and has not been withdrawn from marketing for any reason relating to the safety and effectiveness of the device.[2] Additionally, FDA has accepted toxicological results of earlier silicones with properties comparable to newer versions as relevant for establishing material safety.[2]

The Summary of Safety and Effectiveness for another FDA approved female sterilization device, the Falope Ring® Band, contains reports of long term (78 and 104 week, and lifetime) animal studies on its implanted silicone elastomer. These studies provided no evidence of direct carcinogenic activity of the implanted device. This product has been on the market since 1974 and has not been withdrawn from any market for any reason relating to the safety or effectiveness of the device.

Other devices have used comparable silicone elastomers as well. The Mentor Corporation market a penile inflatable implant with silicone elastomer parts (P000006). The SS&E for this product reports a pharmokinetics assay of silicone monomers and polyurethane products as well as chronic toxicity/carcinogenicity assays that reveal that the product does not pose a toxicological concern. This product was approved by FDA in July, 2000; it has been on the market since 1989 and has not been withdrawn from marketing for any reason relating to the safety and effectiveness of the devices. Finally, the Femsoft Insert® utilizes a silicone patient contact material and reports that acceptance criteria for biocompatibility requirements have been met. The product was FDA approved (P990002) on September 30, 1999.

Extensive published and unpublished toxicology testing results indicate that the silicone materials previously supplied by [redacted] and those currently supplied by [redacted] or hypothetical contaminants thereof, do not produce a positive response in any of the proposed biological tests.[5] Extensive testing on siloxanes (shown to be present at very low concentrations in some silicone elastomeric formulations) also showed no significant genotoxic capability[2].

[redacted]

Therefore, this material falls into the category of "materials known to show no genotoxicity, moreover, all major components of extracts can be identified and have been shown to have no genetic toxicity"[6]. This information is sufficient to justify the deletion of additional genotoxic tests.

4.1.2.6. Conclusions

The Adiana System Delivery Device with Matrix in place, after exposure to steam sterilization cycle, meets the biocompatibility requirements per ISO 10993-1.

The Adiana Matrix after exposure to steam sterilization cycle, meets the biocompatibility requirements for Cytotoxicity, Rabbit and Clinical Implantation and Genotoxicity. Additionally, solid, fully cured silicones such as [redacted] have shown no significant genotoxic capability and have been determined to be non-carcinogenic.

The biocompatibility testing conducted on the Adiana Transcervical Sterilization System demonstrates that the delivery catheter and matrix are biocompatible.

4.1.2.8. Data Records

Manufacturing lot numbers are reported on the original test reports from the test lab, provided in the appendices to the final report.

Manufacturing lot records, including sterilization records, are stored in Quality Department according to the requirements of [redacted] Record Retention.

[6] ISO 10993-3; 1992. *Biological evaluation of medical devices - Part 3: Tests for genotoxicity, carcinogenicity and reproductive toxicity.*

IV. C. VALIDATIONS

IV. C. 1. DELIVERY CATHETER AND MATRIX

- Engineering/Bench Testing

Engineering / Bench testing of the Adiana Transcervical Sterilization System has been conducted in order to verify that the design output conforms to the design input requirements described in the product specification. Description and results of bench testing are reported in full in Section 4.2 for the Adiana Delivery Catheter and Matrix (for both the "Current" and "Original" handles). Per agreement with FDA, testing for the RF Generator will be reported in Module #2.

While Material Biocompatibility is addressed in Section 4.1 of this Module, additional information describing patient contact materials is provided in the Material Analysis Section 4.2.2. of the Engineering/Bench Testing section. Product specifications and inspection criteria are described for each patient contact material including Silicone [redacted] Stainless Steel, [redacted] Tubing, Extrusion [redacted] Composite [redacted] black; and Lubricious Coating.

Verification test results for the Current Delivery Device [redacted] that were submitted as [redacted] are presented in Table 1.2 below and are described more fully in Section 4.2.3.1 (Engineering/Bench Testing).

[redacted]

Test	Units Tested	Results:
[redacted]		

All results obtained during this evaluation were acceptable. The results verify that the design output conforms to the design input requirements described in the product specification.

Additionally, two minor interrelated post-trial device modifications to the "Current" Delivery Catheter are described in Sections 2.6.2 and 4.2.3.2. These changes were developed in response to device malfunctions in the field. The band spacing modification and change in shape to the tip of the push rod are both within original product specifications.

Section 4.2.3 of Engineering/Bench Testing also contains description and verification testing for the "Original" Delivery Device and Matrix (Section 4.2.3.4). A description of the Compression Testing that was performed separately on the Matrix as part of the original Design Verification testing is included (Section 4.2.3.5). Finally, a summary of the 5-Day Notice, submission of minor changes to the "Original" Delivery Catheter [redacted] is provided (Section 4.2.3.2.).

IV. C. 2. RF GENERATOR HARDWARE

6.4 Radio Frequency Generator Hardware Verification

6.4.1 Engineering/Bench Verification Testing

RF Generator testing was conducted on RF Generator assemblies to verify that all requirements specified in the hardware requirement specification are met. Measurements, recordings, observations and processes were used with equipment calibrated to NIST traceable standards. The testing was performed initially in 2001 when the RF Generator was developed. RF generator verification and validation testing were submitted to FDA in support of IDE G020172 in July 2002.

Verification test criteria were developed using several methods. Some measurements were dictated by clinical use requirements. Other tests used criteria which were generated on the basis of prior investigations, including clinical and/or in vitro studies.

Equipment used during these evaluations for test, inspection, and measurement was appropriately calibrated and/or maintained. Description of Stellartech's quality, manufacturing, and preventive maintenance procedures will be provided in Module #3.

Test and inspection procedures and reports for the current RF Generator are contained in [redacted] Hardware Verification Protocol [redacted] (See Appendix 6) and [redacted] Supplemental Hardware Verification Protocol [redacted] (See Appendix 7). Data were recorded during the testing; results were recorded directly on the protocol documents and data sheets are maintained in Quality Records [redacted] and at Adiana. Copies are included with the protocols and results.

During recertification to IEC 601 in late 2005, it was discovered that the auxiliary footswitch used on the RF generator was not IEC 601 compliant. To comply with the IEC 601 specification, a redesign was undertaken to utilize a compliant circuit; a pneumatic footswitch which actuated an internal switch was selected to avoid a non-isolated circuit outside the RF generator enclosure. The footswitch and interface to the RFG were redesigned by Stellartech, and the hardware verification and validation testing was revised. The following changes were made to the generator to achieve compliance:

- Change to a pneumatic footswitch
- Addition of a pneumatic module inside the RF Generator
- Change from an electrical connector to a pneumatic connector
- Changes to the Operating System hardware to sense this signal

These changes were also described in PMA Module 1.

6.4.1.1 Sample Size

One RF Generator was tested for this protocol because this testing is "type testing" of the design, as opposed to reliability testing in which multiple units need to be tested to demonstrated a reliability level at a specified confidence.

6.4.2 Testing Protocol and Results

Verification testing of the RF Generator, performed at Stellartech Research, is summarized in Table 6.2 below. All testing was performed with 115 VAC mains configuration unless otherwise specified. The sequence of testing was the same as the order of testing presented in the Table 6.2 below.

[redacted]

Test	Acceptance Criteria	Results	Reference
RFG Verification Testing (March 2001)			
[redacted]			

Test	Acceptance Criteria	Results	Reference
[redacted]			

Test	Acceptance Criteria	Results	Reference
[redacted]			

Test	Acceptance Criteria	Results	Reference
[redacted]			

All results obtained during this evaluation were acceptable. The results verify that the design output conforms to the design input requirements described in the product specification.

IV. C. 3. RF GENERATOR SOFTWARE

7.9 Verification and Validation Documentation

Software Verification and Validation have been performed by [redacted] pursuant to the FDA Guidance, "General Principles of Software Validation: Final Guidance for Industry and FDA Staff" (January 11, 2002).

The first Software Verification performed for RF Generator preclinical and clinical use was software Version 1.06. This version was submitted as part of the original IDE. [redacted] The purpose of the Verification Protocol was to show that the software behaved as intended. The Software Verification Protocol was written against the Source Code using the Software Design Specification (SDS) as a guideline. Conclusion of the Verification Testing confirmed that SDS requirements had been met. (See Software Verification Protocol & Results [redacted] Appendix 11). Software Version [redacted] was validated through V1.06 Protocol and Validation Results: [redacted] Appendix 12). The test results confirmed that SRS requirements had been met.
In response to clinical observations, changes were made to the software. These changes are detailed in Appendix 13, and included corrections to error handling (i.e. '0L','3E', '2E', & '5A' errors), data storage refinements, a tightening of the contact resistance spec for the PDA tissue contact sensor, and improvements to the matrix release audible tone. The next modified version V1.07, was validated and was submitted to FDA [redacted] Documentation of Verification of Software V1.07 is contained in the Incremental Software Release V 1.07 [redacted] See Appendix 13). The data derived from this protocol indicated that the software behaved as delineated in the same document.

Version 1.08 modifications to the RF Generator software were made to accommodate the new handle matrix release contact. Additionally, based on clinical feedback, the RF Generator software was modified to display the elapsed treatment time in the event of a treatment interruption. Software Version 1.08 incorporated these changes and was submitted to FDA [redacted] Documentation of Verification and Validation of these changes is contained in Stellartech Incremental Software Release V 1.08 [redacted] See Appendix 14). Test results confirmed that SDS requirements listed in the same document were met. Software Design Specification and Verification Protocol and Results [redacted] Appendix 10) confirmed that SDS requirements listed in the same document were met. Software Requirements Specification and Validation Protocol & Test Results V 1.08 [redacted] Appendix 9) confirmed that all software validation test results met the desired criteria.

IV. D. PRIOR STUDIES

IV. D. 1. BACKGROUND AND RATIONALE

2. PRIOR STUDIES – CLINICAL STUDIES

2.1 Background and Rationale

The most common form of contraception in the United States is female sterilization.[8] Annually 600,000 to 700,000 women undergo sterilization procedures.[8] One form of female sterilization involves a mini-laparatomy and a tubal ligation technique which was developed nearly a century ago.[30] Other more common methods utilize laparoscopic techniques which include electrocoagulation or the application of clips or rings to the fallopian tubes.[30] Surgical sterilization procedures have significant disadvantages including equipment costs, facility requirements including resuscitation capabilities, anesthesia, and post-operative pain. Each method of surgical sterilization has method-specific limitations of safety and efficacy.

Due to the limitations of current surgical methods for sterilization, several investigators have proposed alternative methods for transcervical sterilization. Unlike tubal ligation, which often requires general anesthesia and multiple-day recovery times, it was believed that these new techniques might be performed as an outpatient procedure under local anesthesia. Other potential benefits include reduced pain, shorter recovery time, fewer complications, and reduced cost.

Significant research into transcervical methods of sterilization began in the 1970's. Research into several methods continues to the present day. Several of the most significant attempts to develop a safe and acceptable method for transcervical sterilization are discussed below.

Beginning in 1970, several studies were performed involving the blind, transcervical delivery of quinacrine, a sclerosing agent, to the uterus.[22] The quinacrine causes damage to the intramural portions of the fallopian tubes. The damage is sufficiently severe to result in permanent scarring of the fallopian tubes in a high percentage of women. Quinacrine had been delivered transcervically both as a slurry and in pellet form. The most common, recent method of female sterilization with quinacrine involves two transcervical insertions of quinacrine pellets approximately one month apart.[22] Preliminary results of Chilean studies of quinacrine show a 5-year cumulative pregnancy rate of approximately 9 per 100 women for women under age 35 and 3.4 per 100 women for women over age 35.[22] Quinacrine is still being studied for female sterilization (primarily in developing countries).[11] Concerns regarding its safety, e.g. mutagencity and carcinogenicity, and efficacy have limited the acceptance of quinacrine.[11, 22]

Quinones, et al, began investigating hysteroscopic sterilization with electrocoagulation using monopolar radiofrequency current in 1972.[17] The method used by Quinones involved fluid distention of the uterus, transcervical delivery of a unipolar electrode, and the application of coagulating radiofrequency current.[17] The

electrocoagulation was intended to effect occlusion of the tube through tissue scarring which followed this extensive thermal injury. The parameters for the radiofrequency current were approximately 25 Watts for 4 to 8 seconds.[18] In a study of 800 patients, Quinones reported no complications due to anesthesia, distention fluid, or the radiofrequency current.[18] However, the pregnancy rate for the Quinones study was 8.4%.[18] Quinones sited several factors which contributed to the failure rate: procedures were not all coordinated with regard to time of menstrual cycle, investigators were not all proficient in hysteroscopy, and insufficient duration of radiofrequency current application.[18] In addition to pregnancy complications, Quinones reported 1 uterine perforation ascribed to investigator lack of experience and one death which may have been caused by bowel damage during radiofrequency current application.[18]

In the 1980's, transcervical sterilization was attempted using a hydrogelic occlusive device.[3] This implanted device, call the P-block, was studied over a period of ten years and included over 900 woman years of experience.[3] The P-block technique involved distention of the uterus and hysteroscopic delivery of the implanted device (via forceps, and later, a delivery catheter).[3] The hydrogelic body of the P-block device swelled after placement to fill the fallopian tube.[3] The P-block device also featured "retention wings" to prevent its expulsion. Brundin reported that, over time, fibroblastic and vascular ingrowth could occur into the porous surface of P-block device.[3] Studies reported by Brundin indicated that pregnancies occurred due to P-block expulsion (4.6 per 100 woman years) and that some pregnancies occurred despite bilateral retention of the implanted devices.[3] Brundin reported a very low rate of complications, e.g. bleeding, associated with the use of the P-block device.[3] Only one ectopic pregnancy was observed in the studies conducted. Despite significant issues with the performance of the device, there was a high rate of patient satisfaction with the P-block contraceptive method as reported by Brundin.[3]

In the early 1980's, Neuwirth et al, reported on studies involving the transcervical delivery of a tissue adhesive to occlude the fallopian tubes.[15] Methyl cyanoacrylate (MCA) was injected into the fallopian tubes using an intrauterine balloon to drive the adhesive into the isthmic portion of the tubes.[14] The MCA caused inflammation, necrosis, and fibrosis in the fallopian tube over time which was intended to occlude the fallopian tubes.[15] Neuwirth reported on single treatments (single injection) and a two-injection sequence in which a 30-day interval passed in between treatments.[15] Using hysterosalpingography, Neuwirth observed 75% bilateral occlusion in the single treatment cases and 87% bilateral occlusion in the two-treatment cases.[15] Neuwirth reported 23 confirmed pregnancies in the 1,083 women treated with MCA.[15] The observed pregnancy rate was approximately 1 per 100 woman years.[14] As reported by Neuwirth, no pregnancies occurred after 6 months of follow-up and no ectopic pregnancies were observed.[15] During the studies, a complication rate of approximately 1% involving hospitalization for symptoms compatible with pelvic inflammation was observed.[15] The need for multiple treatments and the limited efficacy rate reported were among the limitations of this method.

In studies which continued into the 1990's, a silastic tubal plug was investigated to achieve female sterilization.[27] The treatment involved the hysteroscopic approach to the fallopian tubes, distention of the uterus, and delivery of catalyzed liquid siloxane rubber to each fallopian tube via a catheter.[27] The fluid was allowed to flow to the oviduct, thereby forming a tubal cast which was smallest at the isthmic portion of the tube and larger at either end.[20] The solid silastic cast was intended to achieve sterilization by forming a mechanical occlusion of the tube.[25] (It is important to note that unlike the Adiana treatment, in which the implant is intended to provide a scaffold for a biological block, the solid silicone was intended to form a silicone plug.) Van der Leij reported that proper plug implantation was achieved in 82.7% of patients.[25] In the remainder of patients with proper plug implantation, 3.6% were advised by investigators to not rely on the plugs for contraception because of uncertain status, e.g. damaged plug.[25] A total of 324 patients were allowed to rely on the silastic plugs for sterilization.[25] In this group, the pregnancy rate was approximately 2.3% which included one ectopic pregnancy.[25] Also, there were seventeen additional device-related terminations, nine expulsions and eight removals.[25]

More recently, Kerin, et al, have reported on the use of a metal and Dacron coil placed partially into the fallopian tube. [10] This device creates an inflammatory response which, over time, alters the normal tubal architecture and leads to occlusion and pregnancy prevention. Published results show high efficacy rates, but reports of placement difficulties (a bilateral placement success rate of 84.2%) and complication rates are issues.[25] In addition, this device is much larger than other implants, such as the technique proposed in this PMA, and it is unknown to what extent size and the use of a metal implant will be ultimately accepted by patients and physicians.

On the basis of this prior work, Cytyc began development in 1997 of a new technique for sterilization that would overcome some of the demonstrated shortcoming of prior attempts discussed above. The criteria identified in this development work included:

- Delivery of the device should be achievable without extraordinary training or effort for the physician, and without discomfort or risk to the patient.
- Successful placement should be accomplished in a large proportion of patients attempted.
- The device must result in occlusion of the fallopian tube, as measured by stringent assay.
- The device must lead to a stable tissue response which is indicative of long term efficacy.

The Adiana Procedure involves: 1) hysteroscopic, transcervical introduction of a catheter, 2) hysteroscopically controlled insertion of the distal tip of the catheter device into the fallopian tube lumen, 3) bipolar radiofrequency current application to

the tubal lumen, and 4) implantation of a biomaterial matrix or tissue scaffold in the region of the radiofrequency lesion within the fallopian tube, and 5) ingrowth of healthy tissue into the implant, and incorporation of the implant, to create a long-term blockage.

The Adiana Transcervical Sterilization System (the Adiana System) consists of the Adiana Delivery Catheter with Adiana Matrix, and the Adiana RF Generator.

The underlying mechanism proposed consists of the following components: Radiofrequency current destroys the epithelial lining of the fallopian tube lumen, and stimulates a wound healing response. A matrix has been designed to provide a suitable architecture for tissue ingrowth during this wound healing response. The wound healing response into this unique tissue scaffold results in a complete integration of healthy tissue into the implant. The action of the matrix is not simply to mechanically occlude the tube, but rather to create a tissue scaffold leading to occlusion through the natural healing response.

2.2 Overview- Prior Investigations

Over the course of the last nine years, Cytyc has performed an extensive series of investigations to characterize the safety of the Adiana Procedure, to determine the proper device configuration and to define clinical protocol to achieve tubal occlusion. This research has focused on all aspects of device performance, including access to the fallopian tube, radiofrequency lesion creation, matrix delivery, cellular ingrowth, tubal occlusion and pregnancy prevention.

These studies, some of which have been presented in detail in prior submissions [redacted] are presented in Sections 2.3 through 2.8, are defined below (Note: Details on In Vitro and Animal studies are NOT repeated herein, however salient conclusions are reviewed to provide context for latter studies):

In Vitro Studies [redacted]

These studies utilized extirpated uteri from patients undergoing elective hysterectomy. Following removal, the uteri were used to evaluate catheter handling and performance. In addition, the ability to deliver RF energy and create a superficial thermal injury to the epithelial layer in the fallopian tube was evaluated. Histological analysis following treatment of in vitro samples was used to define proper RF treatment parameters.

Animal Studies [redacted]

The Adiana System was evaluated in the rabbit model. This model was used primarily to assess: acute RF performance, tissue ingrowth into the matrix, fallopian tube blockage and pregnancy prevention. Extensive animal studies have confirmed that the Adiana System will result in tubal occlusion as demonstrated by rigorous challenge with pressurized fluid. In addition, mating studies performed in rabbits have shown pregnancy prevention at 6 months and 12 months post implant.

Peri-Hysterectomy Studies [redacted]

Patients receiving elective hysterectomy were treated with the Adiana System immediately prior to hysterectomy. These studies showed that controlled lesions could be obtained reproducibly, that treatment with the Adiana delivery catheter caused no risk to adjacent organs or structures, that there was no risk of thermal injury during treatment and that the matrix delivery was reliably obtained.

Pre-Hysterectomy Studies

Pre-Hysterectomy studies involved the treatment of hysterectomy patients 6 to 12 weeks prior to their planned hysterectomy procedure. Multiple device configurations were investigated during the course of several Pilot Studies. Once a final treatment protocol and matrix configuration were selected, a series of patients were evaluated in the Pivotal Pre-Hysterectomy Studies. The results of these studies show that the Adiana System occludes the fallopian tube within a period of 12 weeks based on clinical hysterosalpingography (HSG), a rigorous *ex-vivo* pressurized fluid assay ('retrograde salpingography' or RSG) and histological assessment.

Access Studies

This clinical protocol was performed with patients seeking elective tubal ligation. The Delivery Catheter was utilized to access the fallopian tube immediately prior to a scheduled tubal ligation. In this study, no RF energy nor matrix delivery was performed. This study was designed solely to evaluate the ability of the Delivery Catheter to access the fallopian tube in a patient population which was similar to the target population for this device. The results of this study have shown that the Adiana Delivery Catheter has a high access rate in a population of patients seeking tubal ligation.

In all studies involving treatment of human subjects, local ethical committee or IRB approval was acquired. Approvals from the relevant foreign agencies were obtained for studies performed outside the USA. Informed consent was obtained prior to enrollment.

The laboratories where these studies were performed were not GLP certified, however compliance with the essential elements of the GLP requirements (21 CFR Part 58) was achieved. Written procedures and protocols were implemented, and all data and results were subject to review and audit by Cytyc Quality Assurance department.

IV. D. 2. IN-VITRO AND ANIMAL STUDIES

2.3 In Vitro Studies

2.3.1 Background

Cytyc undertook a series of In Vitro experiments to assess the feasibility of the Adiana procedure. These studies were done under Cytyc approved protocol [redacted] (submitted in PMA Module 1). The objectives of this study were three fold:

- To evaluate the safety of using RF energy for ablation of the epithelium within the intramural portion of the fallopian tube, and
- To evaluate the immediate tissue response to various levels of RF energy, and
- To evaluate different delivery catheter and electrode configurations.

These studies were performed with several different catheter configurations during the early portion of the development process. Ultimately, subsequent testing (both In Vitro Studies and later clinical studies) identified the preferred configuration for the Adiana System.

After elective hysterectomy, each extirpated uterus was prepared for introduction of the Adiana Delivery Catheters. This was done either by partial dissection to allow direct access to the fallopian tube, or by standard hysteroscopic technique on the intact organ. Once the Adiana Delivery Catheter was in position, RF energy was applied.

After application of energy, the treated portion of the fallopian tube was excised and processed for histological examination.

Histological examination included either Formalin or Cryo- preservation. Formalin fixed samples were stained with H&E and were taken at [redacted] Cryopreserved samples were processed [redacted] increments and were stained with nitroblue tetrazolium (NBT), a vital stain commonly used to analyze cellular viability. When using NBT stain, cells which are viable stain a blue/purple color and those with denatured respiratory enzymes, the first indicator of irreversible cell death, do not stain.

In either case, the fallopian tube was evaluated for the following:

- Uniformity and completeness of epithelial destruction
- The depth and uniformity of the thermal lesion
- The tubal lesion length, and
- The placement of the electrode array into the tube

In addition to the histological endpoints for this study, serosal temperature monitoring was performed in some studies to evaluate the potential for risk in future in-vivo studies. Serosal monitoring was utilized when a unique catheter configuration and/or RF treatment condition was first evaluated.

2.3.2 In Vitro Results - Early Results

The details of these early initial studies are not presented in detail, however during the course of device testing, the following was observed:

- A 4 electrode bipoloar RF electrode array, 6mm in length and 0.47 inches in diameter was optimal, in terms of tubal access and lesion formation.
- A temperature of 64°C for 60 seconds yielded reproducible superficial lesions within human fallopian tubes.
- Use of a viability stain, nitroblue tetrazolium, was superior to H&E staining to differentiate between living and dead or 'dying' cells. (Although H&E is appropriate to visualize high temperature tissue effects, NBT staining is more sensitive to low level heat effects created with the Adiana system.)

2.3.3 In Vitro Results at final time/temperature parameters (64°C/60 Sec)

It is important to note that development work is typically not a linear process, and this applied to the efforts at Adiana. The In Vitro studies described above were not all performed prior to other work, but were iterative with other clinical studies. Indeed, results of Peri-Hysterectomy studies often led to a re-examination of device configuration or treatment parameters, and additional in vitro work. Ultimately, Adiana chose one single device configuration and RF control parameter setting with which to proceed into human clinical testing.

The results in this section are those obtained with an electrode configuration of 4 electrodes, in a bipolar configuration, 6mm in length and 0.047 inch (1.5mm) in diameter. The Temperature/Time settings used were 64°C for 60 seconds. (This is the same electrode configuration and Temperature/Time parameters as is being used in the current Adiana System.)

2.3.4 Conclusions

On the basis of extensive In Vitro studies (as previously described in Module 1), Adiana determined the following:

- Treatment with the Adiana Delivery Catheter electrode array at 64°C for 60 seconds is safe: The lesion is shallow, does not extend to the serosal surface, and causes no significant serosal temperature rises.
- This treatment yields a high degree of epithelial ablation (93%), a lesion length (5.1mm), and a lesion depth which is relatively shallow (0.514mm). Lesion creation is uniform and reproducible.
- Delivery of the Adiana Matrix within the lesion is feasible in this model.
- The use of a lubricious coating, as defined in Adiana's manufacturing procedure, has no impact on lesion formation
- The use of the Adiana RF Generator is equivalent, in the in vitro model, to the use of the [redacted]

2.4 Animal Studies - General

2.4.1 Overview:

There were two different protocol types which were employed in the development of the Adiana Transcervical Sterilization System:

> ***Short term studies*** designed to evaluate ingrowth characteristics. These studies generally lasted 3 weeks, with an endpoint of histological ingrowth as well as tubal occlusion. Other short term studies included acute studies to evaluate RF lesion creation. These RF lesion studies had no implantation period, but were assessed histologically.
>
> ***Long term fertility studies*** to evaluate the ability to occlude fallopian tubes AND to prevent pregnancy over extended time periods. These studies lasted 12 months, with endpoints including pregnancy prevention, histological analysis and tests of tubal patency.

Over the course of Cytyc's development work since 1997, there have been multiple rabbit studies Many of these studies were employed as 'screening' studies as a prequel to performing human studies, A detailed discussion of these results are not presented, however, the animal *fertility* studies which were conducted are significant and are therefore reviewed in detail in the following section.

2.4.2 Animal Fertility Studies

Cytyc performed two rabbit fertility studies using the present matrix and sterilization process. Both studies were 12 months in overall duration (with 6 month mid-point analyses in which half of the animals were sacrificed at 6 months and the other half maintained for the full 12 months). One study is entitled Long-Term Implant; Sterilization Method Evaluation (Adiana Protocol [redacted] and the other Long-Term Implant; Matrix Length Evaluation (Adiana Protocol [redacted]

These study results have previously been presented in both IDE G020172, and in Module 1 of this PMA.

Overall, these two studies were designed to evaluate the ability of the Adiana treatment to provide long term occlusion of the fallopian tubes in order to prevent pregnancy. To permanently occlude a fallopian tube, it is desired that the ingrowth into a matrix be space filling and have long-term

biocompatibility. Long-term retention of the material is aided by choosing a specific architecture and porosity of matrix that promotes vascularized ingrowth of host cells, preventing the formation of scar tissue[19] and/or a fibrotic capsule around the implant[28]. We believe long-term stability will be achieved through benign host integration of the implant. Several characteristics are required for complete integration of the implant. It is necessary that there be an adequate blood vessel support to maintain healthy tissues, that epithelium be removed to allow tissue ingrowth, and that the implant not progress to scarring which could lead to encapsulation or walling off. Analysis of tissue at the end of the study was performed to confirm this mechanism of action.

Conclusions [redacted]

The primary goals of the study were accomplished as no rabbits became pregnant during the study, and the ingrowth characteristics of porous silicone matrices implanted in rabbit fallopian tubes for 6 and 12 months were evaluated and showed healthy, vascularized, and occlusive ingrowth within the Adiana Matrix.

No pregnancies occurred and all tubes retaining a matrix resulted in tubal occlusion as assessed by dye passage and histological evaluation. All but one of the matrices were retained for the duration of the experiment, and the matrix that was not retained was likely due to misplacement at time of implant.

Conclusions [redacted]

This study was designed to evaluate multiple length matrices. At six months the highest ingrowth scores were achieved by the 3.5 mm matrices (the current matrix design). This was due to the lower blood vessel counts of the 2 mm matrices and higher inflammatory cell counts for both the 2 and 16 mm matrices. By 12 months, the differences between the different length matrices had disappeared and all scores were basically equivalent. It appears that any of these lengths of matrices could be used to cause tubal occlusion in the dye test and good ingrowth. At twelve months, all had very good vascular supply, little inflammation and little necrosis.

The primary objective of pregnancy prevention in all implanted tubes and determining ingrowth characteristics of porous silicone matrices implanted in rabbit fallopian tubes for 6 and 12 months was accomplished. No rabbits became pregnant. All 28 implanted matrices were recovered at explant and all tubes were occluded as determined by passing the dye pressure test.

All three matrices, differing in length only, were successful in occluding the fallopian tube with stable, space filling ingrowth, which successfully prevented pregnancy at up to one year.

SECTION BREAK

4.3. Animal And In-Vitro Investigations

Adiana undertook an extensive product development program, utilizing bench testing, as well as in-vitro tissue studies, animal studies, human hysterectomy studies and finally the EASE pivotal trial. Early activities focused on the development of pre-clinical safety and effectiveness data. These studies were designed to accomplish the following:

- Delivery of the device should be achievable without extraordinary training or effort for the physician, and without discomfort or risk to the patient.
- Successful placement should be accomplished in a large proportion of patients attempted.
- The device should result in occlusion of the fallopian tube, as measured by stringent assay.
- The device should lead to a stable, healthy tissue response which is indicative of long term efficacy.

4.3.1. Surgical Procedure and Deployment

The Adiana treatment involves: 1) hysteroscopic, transcervical introduction of a catheter, 2) hysteroscopically controlled insertion of the distal tip of the catheter device into the fallopian tube lumen, 3) bipolar radiofrequency current application to the tubal lumen, and 4) implantation of a biomaterial matrix or tissue scaffold in the region of the radiofrequency lesion within the fallopian tube, and 5) ingrowth of healthy tissue into the implant, and incorporation of the implant, to create a long-term blockage.

The underlying mechanism proposed by Adiana consists of the following components: First, the delivery of radiofrequency current destroys the epithelial lining of the fallopian tube lumen, and stimulates a wound healing response. Then the matrix is delivered into the RF treated segment of the fallopian tube. The matrix has been designed to provide a suitable architecture for tissue in-growth during this wound healing response. Finally, the wound healing response into this unique tissue scaffold results in a complete integration of healthy tissue into the implant. The action of the matrix is not simply to mechanically occlude the tube, but rather to provide a tissue scaffold leading to permanent occlusion through the natural healing response.

4.3.2 Overview of Prior Investigations

Over the course of the last eight years, Adiana has performed an extensive series of investigations to characterize the safety of the Adiana procedure, to determine the proper device configuration and to define clinical protocol to achieve tubal occlusion. This research has focused on all aspects of device performance including access to the fallopian tube, radiofrequency lesion creation, matrix delivery, cellular in-growth, tubal occlusion and pregnancy prevention. These studies, which are presented in detail in Sections 4.3.3 through 4.3.5, are defined below:

In Vitro Studies
These studies utilized extirpated uteri from patients undergoing elective hysterectomy. Following removal, the uteri were used to evaluate catheter performance and the ability to access the tubal ostia. The ability to delivery RF energy and create a superficial thermal injury to the epithelial layer in the fallopian tube was evaluated. Histological analysis following treatment of in vitro samples was used to define proper RF treatment parameters.

Animal Studies
The Adiana system was evaluated in the rabbit model. This model was used primarily to assess: acute RF performance, tissue in-growth into the matrix, tissue tolerance to the Matrix, fallopian tube blockage and pregnancy prevention. Extensive animal studies have confirmed that the Adiana system will result in tubal occlusion as demonstrated by rigorous challenge with pressurized fluid. In addition, mating studies performed in rabbits have shown pregnancy prevention at 6 months and 12 months post implant.

Pursuant to FDA request, the following studies will be provided in Module 4: Clinical Results. An overview is included below to provide a more complete explanation of the clinical development methodology:

Peri-Hysterectomy Studies
Patients receiving elective hysterectomy were treated with the Adiana system immediately prior to hysterectomy. These studies showed that controlled lesions could be obtained reproducibly, that treatment with the Adiana delivery catheter caused no risk to adjacent organs or structures, that there was no risk of thermal injury during treatment and that the Matrix delivery was reliably obtained.

Access Studies

This clinical protocol was performed with patients seeking elective tubal ligation. The Adiana Delivery Catheter was utilized to access the fallopian tube immediately prior to a scheduled tubal ligation. In this study, no RF energy or matrix delivery was performed. This study was designed solely to evaluate the ability of the Delivery Catheter to access the fallopian tube in a patient population which was similar to the target population for this device. The results of this study have shown that the Adiana Delivery Catheter has a high access rate in a population of patients seeking tubal ligation.

Pre-Hysterectomy Studies

Pre-Hysterectomy studies involved the treatment of hysterectomy patients 6 to 12 weeks prior to their planned hysterectomy procedure. Multiple device configurations were investigated during the course of several Pilot Studies. Once a final treatment protocol and Matrix configuration were selected, a series of patients were evaluated in the Pivotal Pre-Hysterectomy Studies. The results of these studies show that the Adiana Transcervical Sterilization System occludes the fallopian tube within a period of 12 weeks based on clinical hysterosalpingography (HSG), a rigorous *ex-vivo* pressurized fluid assay (RSG) and histological assessment.

In all studies involving treatment of human subjects, local Ethics Committee or IRB approval was acquired. Approvals from the relevant foreign agencies were obtained for studies performed outside the USA. Informed consent was obtained prior to enrollment into the Adiana studies.

Animal testing reported in studies [redacted] and other studies reported herein were not in full compliance with Good Laboratory Practices (GLP) regulations as described in 21 CFR Part 58, however compliance with the essential elements of the GLP requirements was achieved. Written procedures and protocols were implemented, and all data and results are subject to review and audit by Adiana Quality Assurance department.

4.3.3. IN VITRO STUDIES

4.3.3.1. Background

Adiana undertook a series of In Vitro experiments to assess the feasibility of the Adiana procedure. These studies were done under Adiana approved protocol [redacted]. The objectives of this study were three fold:

- To evaluate the safety of using RF energy for ablation of the epithelium within the intramural portion of the fallopian tube
- To evaluate the immediate tissue response to various levels of RF energy, and
- To evaluate different delivery catheter and electrode configurations.

These studies were performed with several different catheter configurations during the early portion of the development process. Ultimately, this testing (both In Vitro Studies and later clinical studies) identified the preferred configuration for the Adiana System. All results from all configurations will be reviewed briefly, but only detailed discussions of the results directly applicable to the current configuration will be presented. A complete report of all results is maintained in Adiana document control.

4.3.3.2. In Vitro Study Protocol, Methods and Trial Execution

4.3.3.2.1. Number of Samples:

A total of 69 uteri were utilized in all in-vitro studies, between [redacted]

4.3.3.2.2. Study Location:

[redacted]

4.3.3.2.3. Methods:

After elective hysterectomy, each extirpated uterus was prepared for introduction of the Adiana Delivery Catheters. This was done either by partial dissection to allow direct access to the fallopian tube, or by

standard hysteroscopic technique on the intact organ. Once the Adiana Delivery Catheter was in position, RF energy was applied.

RF energy was supplied by a commercially available RF generator [redacted] or using the Adiana RF Generator (in the equivalency studies described in [redacted] (See Appendix 33). Continuous power and temperature measurements, from both the catheter and the serosal temperature sensor probe (when utilized), were recorded for the duration of the application.

Section 4.3.3.3.2. contains a complete discussion of serosal temperature limits defined by Adiana for this treatment. [redacted] Results from different time/temperature treatments were analyzed separately.

After application of energy, the treated portion of the fallopian tube was excised and processed for histological examination.

Histological examination included either Formalin or Cryo-preservation. Formalin fixed samples were stained with H&E and sections were typically taken at [redacted] Cryopreserved samples were typically sectioned at [redacted] and were stained with nitroblue tetrazolium (NBT), a vital stain commonly used to analyze cellular viability. The NBT stain differentiates viable cells from those cells with denatured respiratory enzymes, by a purple/blue stain. Denatured respiratory enzymes are the first indicator of irreversible cell death, and will not exhibit staining.

In either case, the fallopian tube was evaluated for the following:

- Uniformity and completeness of epithelial destruction
- The depth and uniformity of the thermal lesion
- The tubal lesion length, and
- The placement of the electrode array into the tube

In addition to the histological endpoints for this study, serosal temperature monitoring was performed on some studies to evaluate the potential for risk to surrounding tissues and organs. Serosal monitoring

was utilized when a unique catheter configuration and/or RF treatment condition was first evaluated.

4.3.3.3. InVitro Early Results

There were a total of 12 individual studies applicable to device development which were performed during the course of the In Vitro Studies. The first 5 of these studies utilized different electrode configurations, catheter/electrode diameters, and treatment parameters.

The details of these early initial studies are not presented, however during the course of these studies, the following was discovered:

- A 4 electrode bipoloar RF electrode array, 6mm in length and 0.47 inches in diameter was optimal, in terms of tubal access and lesion formation.

- A temperature of 64°C for 60 seconds yielded reproducible superficial lesions within human fallopian tubes.

[redacted]

4.3.3.3.1. In-vitro Results at 64°C/60 Sec

It is important to note that development work is typically not a linear process, and this applied to the efforts at Adiana. The In Vitro studies described above were not all performed prior to other work, but were iterative with other clinical studies. Indeed, results of Peri-Hysterectomy studies often led to a re-examination of device configuration or treatment parameters, and additional in vitro work. Ultimately, Adiana chose one single device configuration and RF control parameter setting with which to proceed into human clinical testing.

The results in this section are those obtained with an electrode configuration of 4 electrodes, in a bipolar configuration, 6mm in length and 0.047 inch (1.19mm) in diameter. The Temperature/Time settings used were 64°C for 60 seconds. (These

are the same electrode configuration and Temperature/Time parameters as are being used in the current Adiana System.)

Specific In Vitro Studies which evaluated this 'final configuration' include: [redacted] It should be noted that of these, [redacted] were performed only to evaluate the effect of the lubricious catheter coating, and are discussed separately in a following section.

The table below summarizes the results applicable to this final configuration. A discussion of the results, and the methods utilized to determine the results are included following the summary table:

[redacted]

Endpoint	Evaluation Method	Results For Treatments At 64°C For 60 Seconds (+/- SD)
[redacted]		

4.3.3.3.2. Histological Analysis of Lesion Formation - Epithelial Ablation

A measurement of lesion formation is the tubal surface area in which epithelial destruction was achieved. The objective of applying RF energy is to ablate the epithelium, which lines the fallopian tube, without significant destruction of the surrounding muscle and tissue. Removal of the epithelial cells allows the underlying cells to grow into the matrix.

To determine epithelial ablation measurements, each tube was histologically analyzed for the presence of viable epithelium following in vitro treatment. All histological slides within the length of a lesion were individually evaluated to determine the circumference of the lumen and the portion of the lumen with

ablated epithelium. For any given slide, the percentage of the circumference of the tubal lumen which showed ablated epithelium was measured. (Samples processed via Formalin processing and H&E stain were examined for disrupted cells, while cryopreserved samples stained with NBT stain were examined for viability.) For each slide, a percentage (0 to 100%) was calculated. The values for all slides within the lesion length were then averaged to yield a total percent ablation.

A treatment of 64°C for 60 seconds has an average value of 93% epithelial ablation along the length of the lesion. These values ranged from 35 to 100% and had a standard deviation of 11%. This includes data from 37 tubes obtained in 24 uteri.

Lesion Length

A sufficient length of lesion should be created to accommodate the Adiana Matrix delivered by the Adiana Delivery Catheter. The lesion length is determined by looking at the length between the first histological slide of thermal disruption to the last slide showing thermal disruption.

Each histological section along a tube was analyzed for the presence of thermal disruption (for H&E staining) or for viability (for NBT staining), and the length was determined based on the known section spacing. The average lesion length for 64°C for 60 seconds was 5.1 $\pm$ 1.2 mm. This includes data from 37 tubes obtained in 24 uteri. This result showed that the RF treatment created a lesion which would contain the Matrix.

Lesion Depth

Lesion Depth is another key component of the Adiana treatment. While RF treatment should ablate the epithelium, the depth of the lesion should be minimized to prevent necrosis or damage out to the serosa.

Each section within the length of the lesion was analyzed, and the deepest point of cell death from the lumen surface was determined. This is the maximum lesion depth for a single section. This maximum depth was determined for every section over the length of the lesion, and then averaged to provide the Average Maximum Lesion Depth. Histological analysis included both H&E and NBT

staining as described above. Analysis of results with H&E staining was compared to results obtained with NBT. Observed lesion depth was found to be influenced by staining method, with H&E stained samples showing consistently lower depths. Results are presented for NBT stained samples only for this specific measurement.

The average maximum lesion depth for 64°C for 60 seconds was 0.514mm ± 0.097 mm, with a range of 0.334mm to 0.732mm. This included 35 tubes in 22 uteri, all analyzed with NBT staining. It is important to note that this is a very shallow lesion, and is much less than the average thickness of tissue between the intramural portion of the fallopian tube and the uterine serosa for the samples studied. In no case did the thermal lesion extend out to the serosa.

Serosal Temperature

An important safety consideration for delivery of thermal energy into the uterus is the prevention of a significant rise in uterine serosal temperature. The transfer of heat to organs adjacent to the uterus cannot occur without a rise in serosal temperature. Without a rise in serosal temperature the thermal lesion is entirely encased within the uterine body, and therefore the lesion formation within the uterus cannot cause bowel burns or any other thermal disturbances to adjacent organs.

It should be noted that serosal temperatures obtained with extirpated uteri in this in-vitro setting are 'worst case' since there is no heat removal due to blood flow, nor is the organ in contact with other tissue which would act to sink thermal energy.

Background:
Adiana undertook a review of prior work in order to develop a threshold for a serosal temperature limit. Previous studies have shown that cellular damage due to hyperthermia is an exponential function of temperature and a linear function of time.[41,42,43,46,47] While these studies were conducted on tissue cultures, their results are corroborated by *in vitro* and *in vivo* bulk tissue studies.[44,45,48] The results of these studies can be summarized by saying that a significant proportion of mammalian cells die when exposed to approximately 43° C for an hour, and that for every 1° C increase

above 43° C the exposure time required to achieve the same effect is divided by two. Table 4.40 summarizes these findings.

Table 4.40: Cell Death Time-Temperature Relationship

Tissue Temperature (°C)	Rise Above Normal (°C)	Time to Cellular Death
[redacted]		

The implication from the table above is that a safe temperature cannot be specified without also specifying a maximum exposure time at that temperature.

[redacted]

Results:
Adiana performed serosal monitoring in a total of 25 tubes in 18 uteri. The treatment parameters of 64°C for 60 seconds over the course of the study produced a maximum serosal rise of only 3.7°C.

4.3.3.4. Catheter Coating Verification

Based on physician feedback during early studies, it was felt that a lubricious coating would improve catheter handling. Additional In Vitro studies were performed to investigate the effect of the lubricious coating [redacted] on the RF performance of the catheter (In Vitro 10,11,14). In Vitro evaluations were performed to investigate the impact of the coating on the RF treatment. [redacted] Treatment with catheters manufactured with this process demonstrated no difference in RF lesion when compared to uncoated catheters when tested in 5 tubes. (Subsequently, this coating and coating process was utilized in all further studies, including the Peri-Hysterectomy Studies [redacted] and the Pivotal Pre Hysterectomy study.) This coating is utilized on all catheter versions utilized in the EASE trial.

[redacted]

[redacted]

4.3.3.5. Adiana RF Generator Verification

During development work, and early Adiana studies, a commercially available RF generator was used [redacted] Adiana developed an RF treatment algorithm for the Adiana RF Generator that would closely mimic the treatment algorithms produced by the [redacted] device. The verification of the equivalence in the output parameters between these two devices would allow the direct comparison of results obtained with both devices. Complete testing of the Adiana RF Generator was performed in the Hardware and Software validations studies that will be discussed in detail in Module 2. In Vitro studies were performed to verify the equivalence of the new Adiana RF Generator to the [redacted] unit. (In addition, Peri-Hysterectomy studies were also performed, and will be reported in Module 4.) Methods used are as described above in section 4.3.2. Histological analysis was performed to evaluate lesion formation: lesion length, depth and % epithelial ablation were analyzed. The results demonstrated no difference in RF lesion when they were directly compared [redacted] and when the results were compared to results seen using all tubes treated at 64°C/60 sec:

[redacted]

4.3.3.6. Conclusions

On the basis of extensive In Vitro studies, Adiana determined the following:

- Treatment with the Adiana Delivery Catheter electrode array at 64°C for 60 seconds is safe: The lesion is shallow, does not extend to the serosal surface, and causes no significant serosal temperature rises.

- This treatment yields a high degree of epithelial ablation (93%), a lesion of sufficient length (5.1mm), and a lesion depth which is relatively shallow (0.514mm). Lesion creation is uniform and reproducible.

- Delivery of the Adiana catheter into the human fallopian tube is feasible in this model.

- The use of a lubricious coating, as defined in Adiana's manufacturing procedure, has no measurable impact on lesion formation

- Lesion formation, using the Adiana RF Generator is equivalent, in the in vitro model, to lesion formation using the [redacted]

4.3.4. Animal Studies – General

4.3.4.1. Overview

There were two general animal protocols which were employed in the development of the Adiana Transcervical Sterilization System:

- *Short term studies designed to evaluate in-growth characteristics.* These studies generally lasted 3 weeks, with an endpoint of histological in-growth as well as tubal occlusion. Other short term studies included acute studies to evaluate RF lesion creation. These RF lesion studies had no implantation period, but were assessed histologically.

- *Long term fertility studies to evaluate the ability to occlude fallopian tubes AND to prevent pregnancy over long time periods.* These studies lasted 12 months, with endpoints including pregnancy prevention, histological analysis and a test of tubal patency.

Over the course of Adiana's development work since 1997, there have been multiple rabbit studies. A detailed discussion of these results, many of which were employed as 'screening' studies as a prequel to performing human studies, are not presented in detail. However, animal fertility studies are significant and specifically applicable to the human pivotal study, and are therefore reviewed in detail in the following section.

Results from the short term animal studies, and the rationale for using this model, are reviewed below.

4.3.4.2. Animal Model Rationale

The rabbit model has been used extensively for studies of reproductive biology[28] and offers several advantages for evaluation of the Adiana System. The anatomy and morphology of the rabbit fallopian tube is similar to that of the human[24], although it is slightly smaller. In addition, the uterus of the rabbit differs from the human as it has two completely separate uterine horns and cervices. Should a pregnancy occur, this allows the unambiguous identification of the tube in which an occlusion failure occurred leading to a pregnancy, as opposed to the case in humans where a failure in either tube could lead to a pregnancy in the single uterus. The life cycle and gestation of the rabbit is well understood and breeding is straightforward to an experienced rabbit handler.

The rabbit model differs from humans in that it is not possible to place the Adiana catheter using a hysteroscopic approach; a surgical approach is required. In addition, placing devices into the fallopian tube from the uterine horn requires skill since this is done without direct visualization.

4.3.4.3. Implantation Studies – Short Term

4.3.4.3.1. Study Objectives

Animal studies all utilized the same basic protocol. These studies were designed to evaluate the action of the Adiana Matrix and RF treatment, and were not designed specifically to evaluate device delivery or acute catheter performance.

Implant studies were utilized to evaluate the following:

- Matrix material, design and construction
- RF Electrode Configuration
- Catheter Coating Effects
- Sterilization Method effects
- Production validation tests

4.3.4.3.2. Conclusions and Summary of Short Term Implant Studies

Short term rabbit in-growth studies were employed from [redacted], for several purposes. Studies were performed: to finalize a choice of matrix material and internal architecture; to define the effect of different sterilization methods; to explore RF treatment parameters and control algorithms, and also to evaluate treatment with the new Adiana RF Generator. All animal study outcomes relevant to the current device have subsequently been validated in human studies. In particular, these include Peri-hysterectomy, Pre-Hysterectomy studies and ultimately the pivotal EASE trial. (Pursuant to FDA request, all human clinical data will be presented in Module 4.) Detailed results for all animal studies are maintained in Adiana document archives.

4.3.5. Animal Fertility Studies

Adiana performed two rabbit fertility studies using the present matrix and sterilization process. Both studies were 12 months in overall duration (with 6 month mid-point analyses in which half of the animals were sacrificed at 6 months and the other half maintained for the full 12 months). One is entitled Long-Term Implant; Sterilization Method Evaluation (Adiana Protocol [redacted] and the other is entitled Long-Term Implant; Matrix Length Evaluation (Adiana Protocol [redacted]

One protocol was designed to evaluate the matrix length (2mm versus 3.5mm versus 16mm) and the other protocol was designed to investigate the effect of sterilization methods (Moist Heat or 'Steam' versus [redacted]

Overall, these two protocols were designed to evaluate the ability of the Adiana treatment to provide long term occlusion and pregnancy prevention. To permanently occlude the fallopian tube, it is desired that the in-growth be space filling and have long-term biocompatibility. Long-term retention of the material is aided by choosing a specific architecture and porosity of Matrix that promotes vascularized in-growth of host cells, preventing the formation of scar tissue[19] and/or a fibrotic capsule around the implant[28]. We believe long-term stability will be achieved through benign host integration of the implant. Several characteristics are required for complete integration of the implant: Removal of the epithelial layer provides a 'tissue-to-matrix architecture' interface; Implantation of a biomaterial architecture that promotes vascularized and stable ingrowth; selection of a biomaterial architecture should not result in a host rejection response. Analysis of tissue at the end of these animal studies was performed to confirm this mechanism of action.

Protocol Sequence; Fertility Studies [redacted]

The two long-term fertility studies will be discussed in detail below. All long term fertility studies were done under contract [redacted] are recognized experts in rabbit fertility investigation, and have access to appropriate facilities for both acute treatment and long term animal care.

4.3.5.1. Methods and Assays: Both Fertility Studies

These two separate studies utilized essentially identical procedures and methods

4.3.5.1.1. Implant Methods

Adiana Matrices [redacted] were implanted into rabbit fallopian tubes after RF application of 58°C for 60 seconds.

It is important to note that all rabbit studies presented herein utilize a standard temperature/time parameter of 58°C/60sececonds. This is a different temperature/time than was used in subsequent human studies, however the *lesion* is essentially identical in these two cases: Adiana conducted a study in order to correlate lesions in rabbit fallopian tubes to those created in human fallopian tubes. It was determined that a lesion in rabbit fallopian tubes due to a 58°C/60 second treatment was equivalent (in extent (length, depth, % epithelial cell ablation)) to a lesion in human fallopian tubes caused with a 64°C/60 second treatment. All rabbit studies were designed to mimic human responses therefore utilize this standard temperature/time parameter (58°C/60sec). (Data on file at Adiana)

[redacted] The region of the electrodes was marked with a marker suture and the RF applied. The animal probe was removed from the tube and a 3 inch length of polyimide tubing containing the Matrix was inserted into the lesioned region. A mandrel was used to push the Matrix out of the polyimide tubing and into the fallopian tube. The Matrix was repositioned (with gentle manual massage or the mandrel) if

necessary to center within the lesion. The uterus and the abdomen were sutured shut.

4.3.5.1.2. Breeding Attempts

All breeding attempts were performed using proven bucks. In prior work, 5 female rabbits from the same rabbit colony as were used for [redacted] were bred to bucks from the colony as a positive control. All five females were found to be pregnant after the first attempt.

4.3.5.1.3. Endpoint Assays

Breeding and Pregnancy Determination

Rabbits were evaluated for pregnancy prevention after the Adiana treatment with a single breeding at 5 or 11 months post implant. Between 18 to 21 days after the breeding attempt, rabbits were sacrificed and the uterine horns with the attached, treated oviduct were removed from the animal. The uterine horns were visually examined for the presence or absence of fetuses.

Dye Pressure Test

One to two centimeters of the uterine horn proximal to the matrix and the entire oviductal isthmus distal to the matrix, was excised and trimmed of excess tissue. The specimen was cannulated on the distal oviductal segment. The cannula was connected to a syringe containing 0.2% methylene blue in isotonic saline. Fluid was infused into the oviduct until the fluid was observed to reach the distal end of the Matrix. Pressure was maintained at 50 mm Hg for 1 minute and then 100 mm Hg for four minutes. 100 mm Hg was a sufficient amount of pressure to see expansion of the tubal diameter immediately distal to the implanted Matrix.

Figure 4.6: Photograph of a rabbit dye pressure test showing rabbit fallopian tube distended with blue dye at 100mmHg pressure

Previous experiments determined that dye passed through a non-occluded tube at less than 20 mm Hg (data on file). Pressures utilized in the dye pressure test are 5 times greater than that needed to pass an untreated, patent tube. Immediate dye passage through the tube indicates a non-occluded or patent tube. Delayed dye passage may indicate a partially occluded tube or one that became patent due to the hydrostatic pressure. Lack of dye passage indicated a non-patent or occluded tube. After dye pressure testing, the tubes were then placed in 10% neutral buffered formalin.

Histological Analysis

Fallopian tubes were embedded in paraffin and longitudinal step sections were stained with H & E. The slides were histologically evaluated for Matrix retention, matrix position and placement, and the characteristics of the host cellular in-growth. Six parameters were used in assessing the in-growth and deriving the in-growth score. These are:

- Counts of closed vascular structures (CVS) or blood vessels
- Percentage remaining epithelium
- Counts of inflammatory cells
- Qualitative assessments of giant cells
- Existence and degree of necrosis
- Presence and thickness of a fibrotic capsule.

These factors are used in a weighted scoring system resulting in an overall in-growth score for each individual Matrix.

All histological analysis at Adiana is performed using a common, internally derived, protocol. This protocol provides for scoring of in-growth based on specific histological observations and counts. The same protocol is used for both animal in-growth scoring as well as human in-growth scoring. A copy of this protocol is included in Appendix 32.

4.3.5.2. Long-Term Implant; Sterilization Method Evaluation (Adiana Protocol [redacted]

4.3.5.2.1. Study Design and Number of Animals

This study was designed to evaluate the long term efficacy of the Adiana procedure, and to compare the long term efficacy using two different matrix sterilization methods, steam and [redacted]

A total of 11 animals were treated. Five rabbits were implanted for 6 months, and 6 were implanted for 12 months. Note that in each of these two groups, half of the tubes utilized matrices which were treated with a sterilization method [redacted] that is not currently used for the Adiana product. (Moist heat (or Steam) sterilization was the method used for all human clinical studies.)

4.3.5.2.2. Results

<u>Pregnancy and Pressure Test Results</u>

There were no pregnancies in any of the 22 tubes following the Adiana treatment at either 6 months or at 12 months.

Table 4.43: Pregnancy and Pressure Test Results

Number of Animals	Sterilization	Implant Period	Pregnancy?
5	Steam	6 Month	None
5	[redacted]	6 Month	None
6	Steam	12 Month	None
6	[redacted]	12 Month	None

Out of the 22 tubes, all except one were non-patent (occluded) by the dye pressure test at both 50 and 100mmHg. There was one tube in the 12 month group which leaked dye in which a Steam sterilized matrix [redacted] had been implanted. Subsequent histological assessment showed the matrix was missing. It is

believed that this Matrix was misplaced at the initial surgical implant. (A review of the study notes at the time of implantation indicated "because of vessels, hard to see placement – thinks matrix placement as 80% certain"). Although notes like this in the placement record were rare, such a misplacement is not surprising. Unlike implants in the human, where placement of a single catheter through the uterus into the fallopian tube is accomplished via hysteroscopic visualization, in the rabbit it is necessary to navigate from the uterine cavity into the fallopian tube without direct visualization. The operator must rely on shape and tactile feedback within the surgical field, thereby making proper placement technically challenging.

Histological Results

Both groups showed space filling in-growth at each time point with tissue fully occupying the pores. In-growth penetrated to the solid core of the Adiana Matrix. This full occupation of the matrix leads to tubal occlusion in the dye pressure tests.

Histological analysis showed an implant that was well tolerated by the host, with host cellular in-growth that was characterized to include a combination of different cell types: fibroblasts, macrophages, giant cells, inflammatory cells, epithelial cells, and extracellular matrix. There was no evidence of any fibrous capsule formation. Necrosis was slight. Inflammation was low, particularly in the moist heat sterilized group.

Results were analyzed using Adiana's scoring system (Appendix 32), and are included below for each of the treatment groups.