PROGESTERONE BIOAVAILABILITY WITH A PROGESTERONE-RELEASING SILICONE VAGINAL RING IN IVF CANDIDATES

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Abstract
A vaginal ring made of silicone polymers and barium sulfate, and containing 1 g of pure micronized progesterone, was developed for luteal supplementation in women undergoing cycles of in vitro fertilization (IVF). The ring, modeled on the Estring, was designed as a means of providing continuous intravaginal delivery of progesterone. Bioavailability of progesterone in the blood was demonstrated for 24 hours in IVF candidates who had an endogenous progesterone deficiency after treatment with gonadotropin-releasing hormone (GnRH) analogues. After the first 4 h of increasing release of progesterone from the ring (with mean serum levels of 1.39 ± 0.8 ng/ml after 4 h), only a slight increase in serum progesterone levels (with a mean peak of 1.5 ± 0.45 ng/ml after 24 h) was observed during the rest of the test period. Gonadotropin levels were not affected after insertion of the ring. The ring was well tolerated by the patients. The maximum serum progesterone level was lower in comparison with other forms of progesterone application, but it should be sufficiently high, due to the uterine first-pass effect. This study demonstrated that progesterone administration through a silicone ring for luteal support is feasible in IVF treatment. As the vaginal ring is very well tolerated by the patients, these findings may encourage the pharmaceutical industry to design an appropriate progesterone ring for luteal support.

Key words: Fertility; progesterone; luteal phase support; vaginal ring, IVF

INTRODUCTION
Exogenous progesterone supplementation is a well-established method during embryo transfer in standard in vitro fertilization (IVF), as well as in hormone replacement therapy [1]. In IVF embryo transfer, progesterone is administered in order to support the corpus luteum, which may be compromised during the induction of ovulation with gonadotropin-releasing hormone (GnRH) analogues, or during oocyte retrieval [2].

It has recently been shown that intravaginal progesterone administration has many advantages in comparison with oral or intramuscular administration [3, 4]. Intravaginally administered progesterone bypasses the liver, induces the secretory endometrial transformation typical of the second phase of the menstrual cycle, and provides adequate luteal support during an IVF cycle [5]. Vaginal rings containing progesterone or estradiol, or both, have been developed in order to provide a continuous means of delivering these hormones [6, 7, 8, 9]. The Estring is an estrogen replacement system that was designed to relieve vaginal problems and the symptoms of urogenital atrophy that often occur after menopause. When placed in the vagina, the Estring releases estradiol in a consistent and stable manner for 90 days [10, 11].

Using a vaginal ring modeled on the Estring, we replaced estradiol with 1 g of micronized progesterone and evaluated the resulting bioavailability of progesterone in IVF candidates who had already begun ovarian stimulation treatment with GnRH analogues.

MATERIALS AND METHODS
PREPARATION OF THE VAGINAL RING
The design of the vaginal ring was based on the Estring. The rings were formed from tubes consisting of silicone elastomers, silicone fluid, and barium sulfate in our laboratory. Each tube had an external diameter of 8 mm and an internal diameter of 4 mm, differing from the Estring design in this respect. The lumen of each ring was filled with 1 g pure micronized progesterone [12]. The ring was then shaped in a Teflon template (Karl Lettenbauer Ltd., Erlangen, Germany), and the two ends of the tube were fixed together with a silicone stick. After this, the ring was left in the Teflon block under pressure for 24 hours. At the end of the process, the outer diameter of the ring was 57 mm, its cross-sectional diameter was 9.5 mm, and its core diameter was 5.5 or 1.5 mm, respectively. To enlarge the contact surface of the ring, channels approximately 1 mm deep and 1 mm wide were cut into it with a V-shaped linoleum knife (Haas-Kabuco, Ltd., Erlangen, Germany).

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At the beginning of the study, two tube shapes were available. Both had an outer diameter of 57 mm, with a cross-sectional diameter and core diameter of 9.5 and 5.5 or 1.5 mm, respectively. The thickness of the wall was 4 mm or 2 mm. In order to compare the progesterone diffusion rates of the two tube shapes in vitro, the two types of tube were immersed in a shaking water bath. Each ring was immersed in a glass bot-
metrical filled with 250 ml 5% human albumin solution. Each bottle was incubated in a warm-water bath at 37°C and each ring was continuously shaken. The progesterone released into the human albumin solution by passive diffusion was quantified every 4 hours for a total of 48 hours, and once more after 72 hours. The progesterone content was measured by enzyme-linked immunosorbent assay (ELISA; Boehringer, Mannheim, Germany).

**PROGESTERONE RELEASE IN VIVO**

Seven healthy IVF candidates under the age of 40 were included in the study. Controlled ovarian stimulation was carried out in accordance with the ultralong luteal protocol, using a GnRH agonist depot preparation (triptorelin; Decapeptyl-Depot, Ferring Ltd., Kiel, Germany) administered by intramuscular injection for at least 14 days. The effect of the triptorelin was checked by serial assessments of progesterone and estradiol, and the patients were considered desensitized when their serum estradiol concentration was <0.2 nmol/l.

The progesterone ring was positioned intravaginally by a gynecologist at 8.00 a.m. for 24 hours. The ring was pressed into an oval shape and inserted as deeply as possible into the upper third of the vagina. Blood samples were taken through a peripheral-vein catheter (Abbocath-T, Abbott, Ltd., Frankfurt, Germany) once just before the ring was placed (baseline value) and then every 2 hours until 4.00 p.m., and finally once more the next morning at 8.00 a.m. when the vaginal ring was removed. Serum concentrations of progesterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) were measured by ELISA.

**STATISTICS**

Student’s t-test was used for statistical evaluation. The level of significance was set at P < 0.05. All calculations were performed using the Excel or Statistical Package for the Social Sciences (SPSS) programs.

**RESULTS AND DISCUSSION**

**PROGESTERONE RELEASE IN VITRO**

Figure 1 shows the release of progesterone in vitro. No significant differences were found between the two types of ring with regard to the progesterone diffusion rates in vitro. However, for the in vivo experiments, the tube with the thicker wall (diameter 4 mm) was used, as it was easier to manufacture and to insert into the vagina. The linear release of progesterone was approximately 0.34 ng/hour.

**PROGESTERONE RELEASE IN VIVO**

After administration of GnRH agonists in accordance with the ultralong luteal protocol for at least 14 days, only minimal progesterone levels were detected in the patients’ serum; the baseline values with a maximum of 0.3 ng/ml serum progesterone demonstrated low hormone production by the adrenal gland and excluded any additional progesterone production by the corpus luteum or placenta. This finding confirms the postulation by Kiesel and Runnebaum that depot treatment with GnRH analogues leads, after 14 days, to suppression of pituitary LH secretion and subsequent inactivation of the corpus luteum and loss of progesterone production [13]. After the administration of the GnRH agonist, the serum progesterone concentration thus depended mainly on the exogenously substituted progesterone. As expected, the application of the progesterone vaginal rings did not influence gonadotropin levels, which remained at a low and constant level over the test period (LH 1.01 ± 0.04 mIU/ml, FSH 6.07 ± 0.40 mIU/ml) (Fig. 2).

The serum progesterone concentration increased significantly as early as 2 hours after insertion of the vaginal ring and remained elevated until the end of the examination period (Fig. 3). This demonstrates sufficient progesterone absorption by the vaginal epithelium. It appears that progesterone release from the silicone rings increases for the first 4 hours. After this point, a slight increase in progesterone release was noted. The mean peak serum progesterone concentration 24 hours after insertion of the ring was 1.5 ± 0.45 ng/ml (4.77 ± 1.43 nmol/l).

In summary, progesterone levels remained relatively constant throughout the treatment period, with a mean of 1.38 ng/ml (4.39 nmol/l).

The average serum progesterone concentrations in the patients were approximately 30% lower in comparison with the mean serum progesterone concentrations for 90 days achieved with a polysiloxane vaginal ring containing 1 g of micronized progesterone (10–20 nmol/l; 3.14–6.28 ng/ml) [6]. Maruo et al. examined a similar intravaginal ring model consisting of a toroid of silicone elastomer tubing containing estradiol in one half and 1.8 g or 3.6 g progesterone in the other, administered for 6 months. The mean peak serum progesterone concentration with rings containing 1.8 g progesterone was approximately 2.8 ng/ml

![Fig. 1. Progesterone diffusion from the silicone rings (wall thicknesses 4 mm and 2 mm) in 5% human albumin solution.](image-url)
after 16 days, declining to 1.4 ng/ml [7]. Both of these studies demonstrated constant release of progesterone over a long time period.

The 24-hour period of treatment in the present study was too short to provide a basis for a definitive comparison of the efficacy of the present model with that of other methods of administration. In addition, low serum progesterone levels do not precisely reflect the bioavailability of the progesterone. Experimental and clinical data suggest that there is a uterine first-pass effect after vaginal administration of progesterone, with high uterine concentrations despite low systemic exposure [14, 15]. Zegers-Hochschild et al., for example, observed progestational transformation in glands and stroma similar to that in the luteal phase of a normal menstrual cycle, despite low progesterone concentrations (10–20 nmol/l) [16]. Miles et al. demonstrated that despite lower serum progesterone levels, a higher degree of endometrial transformation was observed with vaginal progesterone administration in comparison with intramuscular administration [4]. The vaginal route has therefore now become the best-established method of administering progesterone for luteal support. In addition, it is easy to administer, avoids the liver first-pass metabolism, and has no systemic side effects [9, 15]. In the present study, detection of progesterone in serum after vaginal administration confirmed the release of progesterone from the vaginal ring and its absorption by the vaginal epithelium.

The feasibility of administering progesterone via a vaginal ring, as an attractive treatment option, was confirmed in this study. The vaginal ring was soft, flexible, and well tolerated by the patients. It is easily placed in the vagina by the physician, as well as by the patient herself. In comparison with the intravaginal use of micronized progesterone in the form of tablets and gel, requiring insertion into the vagina at least twice daily, the ring only needs to be placed in the vagina once during the whole treatment period.

The Estring is a safe and highly effective intravaginal hormone-replacement system and is strongly preferred by patients who have previous experience with other forms of administration [17, 18]. It is surprising, therefore, that progesterone rings are not in wider use and that they are not being further developed by the pharmaceutical industry in order to provide luteal support. The present study may stimulate further research on the use of progesterone-filled vaginal rings based on the Estring, as an alternative to other methods of progesterone administration.

REFERENCES


Received: October 2, 2006 / Accepted: April 16, 2007

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