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Comparison of dienogest and progesterone effects on uterine contractility in the extracorporeal perfusion model of swine uteri

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Conflict of interest

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Abstract

Introduction. Endometriosis is associated with hyperperistalsis and dysperistalsis in the uterus, and it has been shown that progesterone leads to a decrease in uterine contractility. The synthetic gestagen dienogest is often administered in women who are receiving conservative treatment for endometriosis, and it may be the treatment of choice. The present study investigated the effects of dienogest on uterine contractility in comparison with the known inhibitory effect of progesterone. **Material and methods.** Eighty swine uteri were examined using an established extracorporeal perfusion model. The uteri were perfused for at least 4 hours with progesterone (P), dienogest (D), or a modified Krebs–Ringer solution as the control group (C), with uterine contractions being measured using an intrauterine microchip catheter. The amplitude and frequency of contractions and the area under the curve (AUC), reflecting overall contractility, were measured at two separate locations (the isthmus and fundus). **Results**. P led to a significant decrease in the amplitude of uterine contractions and to reduced overall pressure (AUC) at the isthmus and fundus. D led to a significant decrease in the amplitude of contractions and overall pressure (AUC) in the area of the isthmus, but the decrease near the fundus was not significant. The frequency of uterine contractions was not influenced by either P or D. **Conclusions**. These results confirm the known inhibitory effect of progesterone on uterine contractility (relative to amplitude of contractions and overall contractility), affecting the whole organ. Perfusion of the uterus with dienogest also led to a general decrease in uterine contractility, similar to the effect of progesterone.

Key words

Uterus, endometriosis, fertility, progesterone, perfusion model

Abbreviations

AUC	area under the curve
GnRH	gonadotropin-releasing hormone
ICP	intra cavity pressure
pCO_2	partial pressure of carbon dioxide
pO_2	partial pressure of oxygen

Key message

Progesterone is administered in conservative treatment for endometriosis and reduces uterine contractility. Perfusion of the uterus with dienogest (a synthetic gestagen) in an extracorporeal swine perfusion model led to a reduced uterus contractility, comparable to the known progesterone effect.

Introduction

Endometriosis, a benign disease, is defined as the presence of endometrial tissue outside the uterine cavity (1). The estimated prevalence of the condition ranges from 2% to 10% (2). Rates of endometriosis in the range of 35–50% are seen in women suffering from pelvic pain or infertility, or both (3). The main symptoms of endometriosis are dysmenorrhea, pelvic pain, and infertility (4). The severity of the pain reported shows a wide range, but without any relationship to the morphological stage of the endometriosis (5). The women affected also show an increase in uterine contractility, and uterine hyperperistalsis and dysperistalsis are typically noted (6). The treatment consists of either surgical removal of endometriotic lesions, or medical treatment using gonadotropin-releasing hormone (GnRH) analogues, progestins, or combined oral contraceptive pills (2,7).

The synthetic progestin dienogest (17alpha-17-hydroxy-3-oxo-19-norpregna-4,9-diene-21-nitrile) was initially used as one of the components in combined hormonal contraceptives. It has been approved since 2010 for the treatment of endometriosis, at a daily dosage of 2 mg (8). Studies have already demonstrated that it leads to a reduction in pain similar to that provided by GnRH analogues in patients suffering from endometriosis, along with a

significantly better side effect profile (8–10). Dienogest is therefore the treatment of first choice and is preferable to GnRH analogues (2,7).

In 1986, Buletti and colleagues reported the first experiments with extracorporeally perfused human uteri (11). In view of the large number of uteri needed for the present study, as well as for ethical reasons, it was not possible to use nonpathological human uteri for this investigation. The extracorporeal perfusion model involving swine uteri has been used by our research group for many years to examine the effects of various substances such as oxytocic drugs (12), tocolytic drugs (13), human seminal plasma (14), estrogen, progesterone (15), and prostaglandins (16) on the uterus and on uterine contractions, by measuring the intrauterine pressure. Physiological conditions can be maintained for up to 7 hours, as has previously been shown (12).

The purpose of the present study was to investigate the effects of dienogest on uterine contractions, using progesterone as the reference substance.

Material and methods

Swine uterus

Swine uteri are often used in research, as they have a number of anatomical and physiological characteristics in common with human uteri. The swine uterus is a bicornuate uterus with a single body about 5 cm long and a single cervix about 15 cm long (17,18). The histological structure of the swine uterus is similar to that of the human uterus. The three elements of the uterine wall are, from inside to outside: endometrium, myometrium, and perimetrium. The myometrium consists of smooth muscle cells oriented in an inner circular layer and an outer longitudinal layer, and it induces the contractions measured in the experiment (17,18). The sow is polyestrous, normally with an estrous cycle of about 20–21 days. This cycle is separated into four stages: estrus, metestrus, diestrus, and proestrus.

All of the swine uteri were supplied from healthy animals on a daily basis from the local slaughterhouse. After the sow has been killed by electric shock, the uterus is separated from the rest of the body within about 2 minutes. The mean weight of the uteri was 122.8 g (range 71.6–159.2 g). The overall condition of the uteri, the condition of the uterine arteries, and the weight of the organ were the most important criteria used to select the uteri. It was not known

in which part of the estrus cycle the animals were in, and this was therefore not a selection criterion.

Perfusion system

After dissection, both uterine arteries were canalized with 24-G needles (Introcan Certo; B. Braun Melsungen AG, Melsungen, Germany) and afterwards the uterus was placed in a temperature-controlled perfusion chamber (Karl Lettenbauer, Erlangen, Germany) filled with perfusion medium (Figure 1). For the first few minutes, each artery was also connected to a reservoir containing the perfusion medium. The perfusion medium used was a modified Krebs–Ringer bicarbonate solution containing NaCl 6.896 g/L, KCl 0.372 g/L, $MgSO_4$+H_2O 0.246 g/L, $CaCl_2$+$6H_2O$ 0.547 g/L, KH_2PO_4 0.136 g/L, $NaHCO_3$ 2.305 g/L, d-glucose 1.5 g/L, saccharose 0.7 g/L, glutathione 0.05 g/L, 1,4-dithiothreitol 0.1 g/L, and insulin 50 IU/L. The pH was checked and adjusted to 7.4. The modified Krebs–Ringer bicarbonate solution was then continued, for the control group; or the swine uteri were perfused with the medium containing progesterone at concentrations of 10 ng/mL, 20 ng/mL or 40 ng/mL; or dienogest at concentrations of 10 ng/mL, 20 ng/mL, 40 ng/mL or 80 ng/mL. Throughout the experiment, the perfusion medium was constantly oxygenated with carbogen gas, a mixture of 95% oxygen and 5% carbon dioxide (Linde AG, Pullach, Germany), the temperature of the medium was constantly 37°C with a continuous perfusion rate of 10-15 mL/min, depending on the intraarterial pressure; mean intraarterial pressure was 60-80 mmHg. A heparin perfusion is normally not necessary to prevent vascular obstruction (11-16).

Vitality parameters

Vitality parameters were analyzed using a Radiometer ABL800Flex analyzer (Radiometer Ltd., Willich, Germany). Perfusate samples were taken at 1-hour or 2-hour intervals, with the following data being measured: pH (Figure 2), pO_2, pCO_2, lactate, and HCO_3, data not shown but were reported previously in detail (12, 13).

Intrauterine pressure measurement

Uterine contractions were measured using a double-chip microcatheter (Urobar DLS-SF; Raumedic AG, Dietzenbach, Germany) placed in an intrauterine location with a distance of 7 cm between the two intra cavity pressure (ICP) sensors. The first pressure sensor was placed in the upper body of the uterus, near the fundus (ICP 1), and the other in the lower body, near the isthmus (ICP 2). After calibration of the system to atmospheric pressure, the

experiment was started. The double-chip microcatheter was connected to a Datalogger (MPR 1; Raumedic) and the data for uterine contractions, measured in real time, were transferred to a personal computer.

Induction of uterine contractions

Regular uterine contractions were induced by administering oxytocin (Syntocinon 10 IU; Novartis Pharma Ltd., Nuremberg, Germany) at increasing dosages of 0.1 IU, 0.3 IU, and 1.0 IU every 15 min. Uterine contractions were recorded for approximately 1 hour. The same procedure was then repeated. Oxytocin was administered as a bolus injection through the uterine arterial catheters. After the first injection of 1.0 IU oxytocin, the perfusion medium was changed, depending on the experimental group: either the modified Krebs–Ringer bicarbonate solution was continued, in the control group, or a Krebs–Ringer bicarbonate solution with progesterone or dienogest at different concentrations was used.

Drugs

The effects of progesterone and dienogest on uterine contractions were investigated. Initially, the two agents were dissolved in dimethylsulfoxide (DMSO) (Calbochem, EMD Chemicals Inc., San Diego, California, USA) and they were then diluted with Krebs–Ringer bicarbonate solution. The solution was forced into the vascular system of the uterus at a constant rate via roller pumps. The following concentrations were tested: for progesterone, 10 ng/mL, 20 ng/mL, and 40 ng/mL; and for dienogest, 10 ng/mL, 20 ng/mL, 40 ng/mL, and 80 ng/mL. Both progesterone and dienogest were provided by Bayer Schering Pharma AG (Berlin, Germany).

Statistical analysis

The Wilcoxon signed rank test was used to evaluate significant differences. Statistical significance was set at $P < 0.05$.

Results

A total of 90 uteri were perfused either with progesterone (P) solution, dienogest (D) solution, or with only the Krebs–Ringer bicarbonate (C) solution in the control group. Eight uteri could not be analyzed due to methodological problems during perfusion. Those uteri

showed no contractions at all or the vitality parameters were out of the range right at the beginning of the perfusion experiment. The number of all perfused uteri is shown in Table 1.

The uteri were perfused for at least 4 hours. During this period, the vitality parameters remained almost within the physiological range (Figure 2). The amplitude and frequency of uterine contractions and the area under the curve (AUC) were analyzed.

Progesterone

Each concentration of progesterone resulted in a decrease in the amplitude of uterine contractions at both measurement sites (ICP 1 near the fundus, ICP 2 near the isthmus) (Figure 3). In comparison with the control group, the decrease near the isthmus (ICP 2) was significant ($P < 0.05$) at all concentrations; the decrease near the fundus (ICP 1) was only significant ($P < 0.05$) at the higher concentrations of 20 ng/mL and 40 ng/mL.

The frequency of uterine contractions did not appear to be influenced by perfusion with 10 ng/mL, 20 ng/mL, or 40 ng/mL of the progesterone solution (Figure 4).

In addition to the amplitude of uterine contractions, the area under the curve (AUC) also showed a decrease at all of the concentrations measured (Figure 5). A significant decrease ($P < 0.05$) in the AUC near the isthmus was detected at all of the concentrations perfused. Near the fundus, only the concentration of 40 ng/mL progesterone showed a significant decrease ($P < 0.05$).

Dienogest

Significant decreases in the amplitude of uterine contractions were measured near the isthmus after perfusion with dienogest 20 ng/mL ($P < 0.01$), 40 ng/mL ($P < 0.01$), and 80 ng/mL ($P < 0.05$), but the decrease did not reach significant levels near the fundus (Figure 3).

No significant changes were observed in the frequency of uterine contractions by perfusion with the dienogest solution in general (Figure 4).

A significant decrease in the AUC was observed near the isthmus at dienogest concentrations of 20 ng/mL ($P < 0.01$), 40 ng/mL ($P < 0.01$), and 80 ng/mL ($P < 0.05$) (Figure 5). No significant differences were detected near the fundus, but a trend toward a concentration-dependent decrease was noted.

Comparison of progesterone and dienogest

Decreases in the amplitude of contractions and decreases in the AUC were observed at all of the measured concentrations for both test substances, reflecting an inhibitory effect of both substances on uterine contractility. However, progesterone caused a significant decrease at all of the measurement sites, while dienogest caused a significant decrease only at the isthmus, while the changes at the fundus did not reach significance. The frequency of uterine contractions was not influenced by either progesterone or dienogest.

Discussion

Women suffering from endometriosis have a wide range of symptoms, such as dysmenorrhea, pelvic pain, and infertility, but 20–25% of them also remain asymptomatic (19,20). Endometriosis is a condition associated with a high morbidity rate, which affects women's physical and also psychological well-being (1).

Various theories to explain endometriosis have been suggested. In the earliest of these, in 1927, Sampson proposed that endometrial lesions might develop in the peritoneum as a result of retrograde menstruation (21). However, the exact cause of endometriosis still remains unclear. More recently, Leyendecker et al. have suggested the theory that uterine microtrauma causes higher levels of local estrogen production, which may cause uterine hyperperistalsis, as a result of which some cells in the basal endometrium are replaced (4,22).

In recent years, the focus has more and more been on a correlation between disturbed uterine contractility and the occurrence of endometriosis. There are two main methods of measuring uterine contractions in humans: transvaginal ultrasonography and the use of intrauterine tip catheters (23).

Physiologically, there are three types of contraction, depending on the menstrual cycle. These are important for the reproductive process. The first type appears during menses. Strong uterine contractions directed from the fundus to the uterine cervix can be detected. During the follicular phase, under the influence of estrogens, there is an increase in uterine contractions directed from the cervix toward the fundus, supporting sperm transport. This is followed by the luteal phase, in which, under the influence of progesterone, fewer uterine contractions

occur and the uterus passes into a relatively inactive phase, supporting implantation (6,24,25-27).

A typical finding in endometriosis is uterine hyperperistalsis and dysperistalsis (6,19, 24,28).

In addition to surgical removal of endometric lesions, there is also the option of medical treatment using GnRH analogues or synthetic progestogens such as dienogest. Due to its chemical structure, dienogest has a special pharmacological profile. It combines characteristics of C-19 nortestosterone and progesterone derivatives (29). Dienogest has an affinity to the progesterone receptor that is approximately 10% of that of progesterone (30). By inhibiting the synthesis of estradiol, dienogest leads to atrophy of endometrial lesions (30). Cosson et al. have shown that postoperative treatment with dienogest is as effective as treatment with GnRH analogues (9). Various studies have also reported that dienogest reduces pain (8,10). One mechanism for the effect of dienogest may be its influence on uterine contractility, with a reduction in hyperperistalsis and dysperistalsis, and thus a reduction in uterine pressure.

The extracorporeal perfusion model of swine uteri was used in the present study to investigate the influence of dienogest, with progesterone as the reference substance. This perfusion model has been used by our research group for many years and allows investigations to be carried out in physiological conditions.

The study again confirms the known inhibitory effect of progesterone in this ex vivo model, as reported previously (15). The amplitude of uterine contractions and the AUC were reduced at all concentrations. With dienogest, maximum inhibition appears to occur at concentrations of 40 ng/mL, while concentrations of 80 ng/mL did not result in any further decrease. The results with regard to the frequency of contractions were not significant for either hormone.

Dienogest also leads to a reduction in the amplitude of contractions and the AUC at all concentrations, in an almost dose-dependent manner. The amplitude of contractions and the AUC declined nonsignificantly only at 10 ng/mL. However, dienogest did not match the inhibitory effects of progesterone at all of the measured sites; the differences only reached significance at the isthmus of the uterus. This might be due to the different receptor affinity of the two substances and their different bioavailability, as reported by Sasagawa et al. (31). Dienogest showed lower receptor affinity in vitro in comparison with progesterone. However,

in vivo dienogest is normally not bound to serum proteins, in contrast to progesterone, and this leads to greater bioavailability of dienogest and similar efficacy in inhibiting uterine contractility (32).

Conclusion

The inhibitory effect of dienogest on the contractility of the uterus was found to be similar to that of progesterone in this study. This study also once again shows that the extracorporeal perfusion model of swine uteri that has been employed by our research group for many years can be used to investigate the effects of different substances on uterine activity in physiological conditions.

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Legends

Figure 1. A swine uterus with arterial catheters placed in the uterine artery.

Figure 2. pH measurements over 4 hours. The data are shown as means plus or minus standard deviation.

Figure 3. The effects of progesterone (left) and dienogest (right) on the amplitude of uterine contractions; showing the concentrations 10 ng/mL, 20 ng/mL, and 40 ng/mL progesterone as well as 10 ng/mL, 20 ng/mL, 40 ng/mL, and 80 ng/mL dienogest, along with the control group. The data are shown as means plus or minus standard deviation. $*P < 0.05$ and $**P < 0.01$ vs control.

Figure 4. The effects of progesterone (left) and dienogest (right) on the frequency of uterine contractions; showing the concentrations 10 ng/mL, 20 ng/mL, and 40 ng/mL progesterone as well as 10 ng/mL, 20 ng/mL, 40 ng/mL, and 80 ng/mL dienogest, along with the control group. The data are shown as means plus or minus standard deviation**.**

Figure 5. The effects of progesterone (left) and dienogest (right) on the area under the curve (AUC); showing the concentrations 10 ng/mL, 20 ng/mL, and 40 ng/mL progesterone as well as 10 ng/mL, 20 ng/mL, 40 ng/mL, and 80 ng/mL dienogest, along with the control group. The data are shown as means plus or minus standard deviation. $*P < 0.05$ and $**P < 0.01$ vs control.

Table 1. Number of all perfused uteri.

P solution		Control	10ng/mL	20ng/mL	40ng/mL	80ng/mL	Sum
uteri perfused	9		9	9	8	0	**35**
drop out	1		1	1	2		**5**
D solution	**Control**		**10ng/mL**	**20ng/mL**	**40ng/mL**	**80ng/mL**	**Sum**
uteri perfused	9		9	9	10	10	**47**
drop out	1		1	1	0	0	**3**

Fig. 1

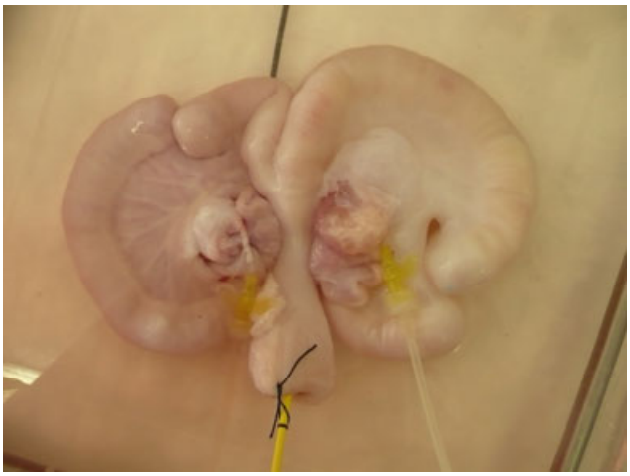

Fig. 2

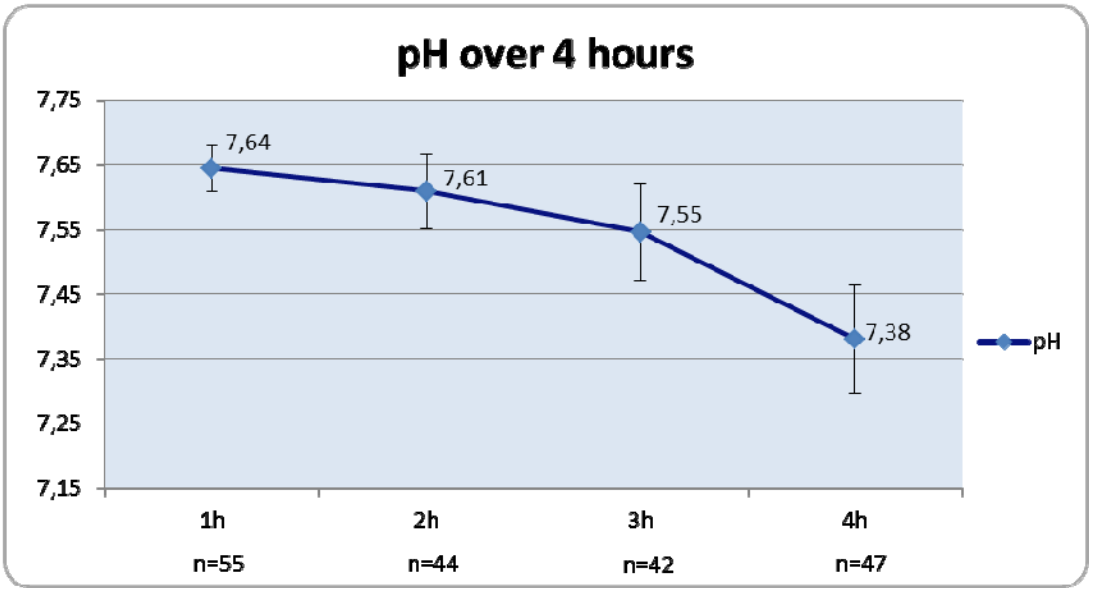

Fig. 3

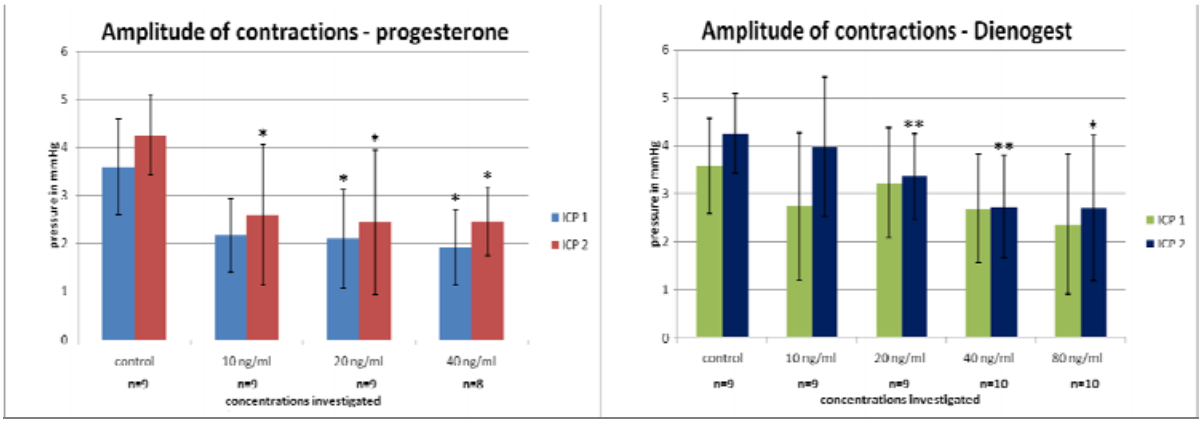

Fig. 4

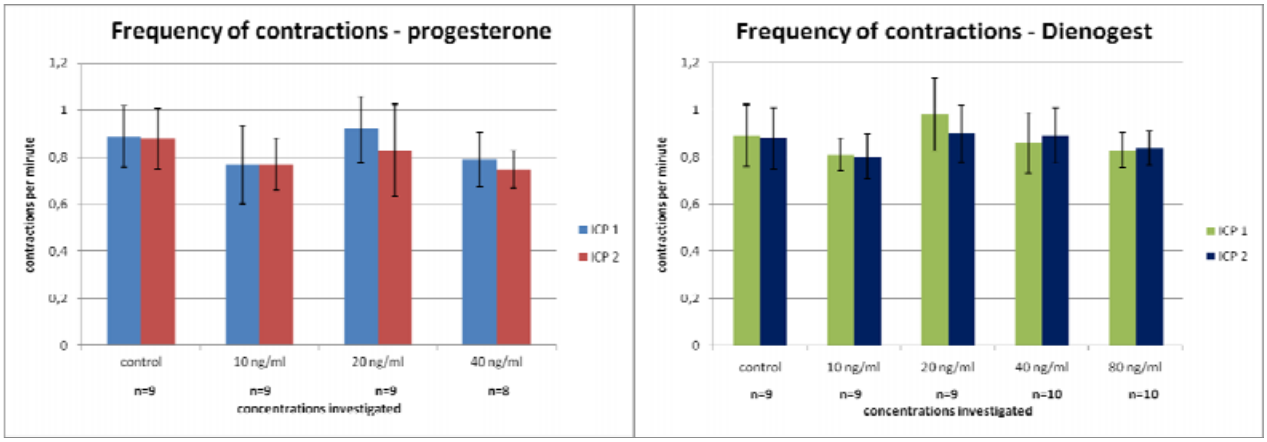

Fig. 5