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**EXTRACORPOREALLY PERFUSED NON-PREGNANT SWINE UTERI: A MODEL FOR EVALUATING UTERINE CONTRACTILITY, PERISTALSIS AND TRANSPORT MECHANISMS**

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**RESEARCH ARTICLE**

**ABSTRACT. OBJECTIVE:** To establish an experimental model of extracorporeally perfused non-pregnant swine uteri for the study of uterine contractility, peristalsis and transport mechanisms through the female genital tract using an intrauterine double-chip microcatheter. In order to validate the model described biochemical parameters were assessed during the whole perfusion time. **METHODS:** An extracorporeal perfusion model of swine uteri was used to keep the uterus in a functional condition and is appropriate for the study of physiological questions. Thirty-two swine uteri were perfused with different concentrations of  $17\beta$ -estradiol simulating the hormone levels during the periovulatory phase and to provide an estrogen-priming of the uteri. Oxytocin-induced uterine contractility and peristalsis was assessed using an intrauterine double-chip microcatheter. **RESULTS:** The vitality parameters remained physiological during the first 8 hours of perfusion. A dose-dependent increase in IUP in the isthmus ( $P < 0.005$ ) and corpus uteri ( $P < 0.005$ ) was observed. The pressure increase was significantly higher in the isthmus uteri than in the corpus uteri at all concentrations tested resulting in a cervico-fundic pressure gradient. In addition, significant more peristalsis started in the isthmus uteri and was moving in the direction of the corpus uteri ( $P < 0.001$ ). **CONCLUSIONS:** The extracorporeally perfusion model of non-pregnant swine uteri kept the uterus in a functional condition and showed a good preservation of the organ for up to eight hours. Therefore this *ex-vivo* perfusion model can serve as an adequate model for studying uterine contractility, peristalsis and transport mechanisms in obstetrics and reproductive medicine and can be used for further *in vitro* experiments.

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## 1. INTRODUCTION

Adequate uterine contractility is involved in the transport of the semen and gametes and in successful embryo implantation, while inadequate uterine contractility may lead to ectopic pregnancies, miscarriages, retrograde bleeding and endometriosis [1,2]. Intact utero-tubal transport function and directed sperm transport due to uterine contractility are therefore of critical importance in human reproduction processes [3-5]. Normally, uterine contraction waves showed the highest frequency and amplitudes during the periovulatory phase, while in the other phase contraction waves with lower frequency and amplitudes are observed. Particularly during menstruation, peristalsis appears to be directed towards the cervix, while during the periovulatory phase peristalsis seemed to be directed to the fundus uteri [6,7]. This periovulatory peristalsis, interpreted as rapid sperm transport to the side bearing the dominant follicle, may be a precondition for successful reproduction in humans [3,4,8,9].

The investigation of uterine contractility and peristalsis and its regulation is of clinical interest not only in obstetrics – e.g., to induce labour or to delay birth and prolong pregnancy – but also in gynaecology and reproductive medicine, especially in regard to transport of the semen and gametes and embryo implantation. The original extracorporeal perfusion model of an isolated uterus was first described by Bulletti et al. 1986 [10]. Previous investigations have validated the feasibility of this perfusion model of isolated uteri for various purposes [11-20]. The development of an extracorporeal perfusion model of the non-pregnant swine uterus enabled our group to carry out general assessments of uterine contractility [21-24]. This perfusion model is able to keep the swine uterus in a functional condition and is suitable for the study of physiological questions [12,14,21]. This experimental system can detect intrauterine pressure changes due to mechanical contractions of the organ, as it maintains the architecture and intercellular relations of the uterus [12]. The advantage of the animal model used is that it makes it possible to test a large number of uteri, similar in size and condition, from

young and healthy animals within the reproductive lifespan. The isolated swine uteri were provided by the local slaughter house, avoiding the costs and responsibilities of an animal breeding unit or doing surgery on the animals. Furthermore using the swine uteri model makes it possible to avoid in vivo testing.

To assess dynamic changes in uterine peristalsis and pressure gradients continuous monitoring of intrauterine pressure using multiple probes at different locations inside the uterus may be a suitable method of evaluating directed uterine peristaltic waves and pressure gradients. A double-chip microcatheter with two pressure sensors, normally used for pressure measurements in the bladder and urethra, was therefore used to assess directed uterine peristalsis. The goal of the present study was to assess directed uterine peristaltic waves and pressure gradients in isolated extracorporeally perfused and estrogen-primed non-pregnant swine uteri and to validate the swine uterus perfusion model to serve as a model for studying uterine transport mechanisms. Furthermore we examined some biochemical parameters which are indicators of tissue ischemia or cell necrosis showing the preservation of the organ due to the perfusion system described.

## 2. MATERIALS AND METHODS

### 2.1. SWINE UTERUS

Swine (*Sus scrofa domestica*) are widely used in research. The swine uterus is a long bicornuate uterus with a single corpus and a single cervix. The uterine wall has a similar architecture to that in humans and in other domestic animals, with the three classic histological elements of the uterine wall — the endometrium; the myometrium, which consists of clearly oriented smooth-muscle cells; and the perimetrium. The endometrium contains many hundreds of glands in a cross-section of the uterine wall, and the myometrium is clearly differentiated into inner circular and outer longitudinal layers. The Fallopian tubes in the adult female have the same diameter as those in humans. However, they are much longer, and the uterine corpus is also longer in comparison with human uteri. The sow

has an estrous cycle of 20–21 days.

Thirty-two swine uteri were obtained from the local slaughterhouse. They all came from healthy animals aged 5–18 months. Swine uteri were selected on the basis of their size and overall condition, as well as the condition of the uterine arterial stumps. The mean weight of all the swine uteri was 113 g (range 82.5–153.8 g). Swine uteri are very easily separated from the rest of the body within approximately 2 min shortly after the animal is killed by electric shock (1.5 A, 400 V, 4 s).

## 2.2. PERFUSION SYSTEM

After catheter placement in the uterine vessels with 16–24-gauge needles (Abbocath-T; Abbott Ireland, Sligo, Ireland), depending on the uterus size, the organ was placed in a controlled-temperature perfusion chamber (Karl Lettenbauer, Erlangen, Germany) filled with the perfusion medium. The uterus was then connected bilaterally with two reservoirs containing the perfusion buffer

(Krebs–Ringer bicarbonate glucose buffer, Sigma, Deisenhofen, Germany). The perfusion medium was oxygenated with carbogen gas (a mixture of 95% oxygen and 5% carbon dioxide) and then forced into the uterine arterial catheters with two roller pumps. Normally the perfusion of the uteri was started 30 min after the animal was killed. The flow rate of the perfusion medium was constantly monitored and kept at 15 mL/min and 100 mmHg. To simulate hormone levels during the periovulatory phase 17 $\beta$ -estradiol (Sigma, Deisenhofen, Germany) was added to the perfusion buffer at concentrations of 1 pg/ml, 10 pg/ml and 50 pg/ml, and 8 swine uteri were perfused with each concentration to perform an estrogen-priming of the uteri.

## 2.3. VITALITY PARAMETERS

Perfusate samples were taken at 1-hour intervals for measurement of pH, PO<sub>2</sub>, PCO<sub>2</sub>, HCO<sub>3</sub>, lactate, and oxygen saturation. The perfusate samples were analysed using an i-STAT portable clinical

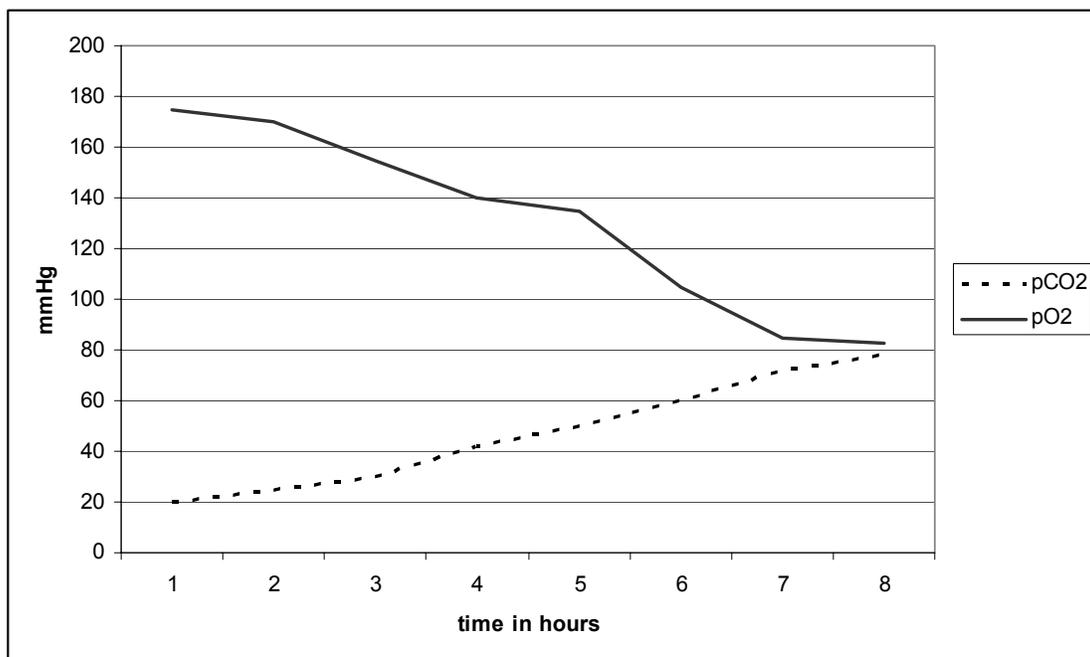


FIGURE 1. ONE EXAMPLE OF PO<sub>2</sub> AND PCO<sub>2</sub> MEASURED IN ONE SWINE UTERUS DURING THE PERFUSION TIME.

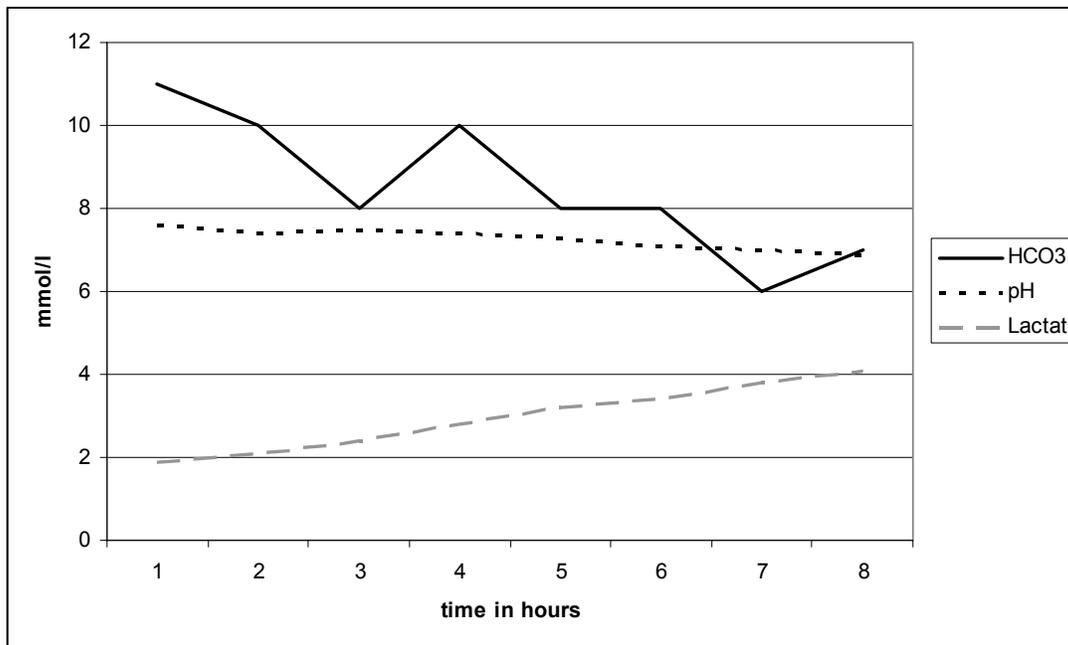


FIGURE 2. ONE EXAMPLE OF HCO<sub>3</sub>, LACTATE AND pH MEASURED IN ONE SWINE UTERUS DURING THE PERFUSION TIME.

analyzer (Abbott Diagnostics, Abbott Park, Illinois, USA).

#### 2.4. INTRAUTERINE PRESSURE MEASUREMENT

Intrauterine pressure was recorded using an intrauterine double-chip microcatheter (Urobar 8/2 DS-F, Raumedic, Muenchberg, Germany) with a distance of 6.5 cm between the two pressure sensors. One sensor was placed in the isthmus uteri and the other was placed in the corpus uteri in the swine uterus. The Fallopian tubes and cervix uteri were not closed. The double-chip microcatheter was connected to a Datalogger (MPR1, Raumedic, Muenchberg, Germany) for continuous monitoring of intrauterine pressure at both locations, with the data being transferred to a personal computer.

#### 2.5. INDUCTION OF UTERINE CONTRACTIONS

Oxytocin (Syntocinon; Novartis Germany Ltd., Nuremberg, Germany) was used to induce contractions of the uterus at increasing dosages of 0.1, 0.3

and 1 IU every 15 min until regular uterine contractions were observed. Oxytocin was administered as a bolus through the uterine arterial catheters. Oxytocin administration was started after 2 hours of initial perfusions containing the above-mentioned concentrations of 17 $\beta$ -estradiol.

#### 2.6. STATISTICAL ANALYSIS

A paired t-test was used for statistical evaluation of IUP increases during uterine contractions in the isthmus uteri in comparison with the corpus uteri for each tested concentration of the test substances. Pressure differences between the different concentrations of test substances were evaluated using analysis of variance (ANOVA) for the repeated measurements. In addition, the chi-squared test of independence was used to assess whether uterine contractions started in the isthmus uteri or the corpus uteri. All calculations were performed using the Statistical Program for the Social Sciences (SPSS, version 10.1 for Windows; SPSS, Inc., Chicago, Illinois, USA). P values of less than 0.05 were



FIGURE 3. TYPICAL PRESSURE PROFILE OF A PERFUSED SWINE UTERUS (THE PERFUSION BUFFER CONTAINS 50 PG/ML 17 $\beta$ -ESTRADIOL). After administration of oxytocin (oxytocin administration = arrow), clearly visible regular peristaltic waves directed from the cervix uteri toward the corpus uteri occurred, demonstrated by an IUP increase first in the isthmus uteri (red curve), followed by IUP increase in the corpus uteri (blue curve). (Y-coordinate = change in IUP in mmHg, X-coordinate = time of day). The green curve is the intra-arterial perfusion pressure as described previously [24], this data were not evaluated for this experiments.

considered statistically significant.

### 3. RESULTS

#### 3.1. VITALITY PARAMETERS

The experiments were only carried out when it was possible to maintain constant flow rates of the perfusion medium of 15 mL/min through each artery, with an ideal basal pressure of 40-50 mmHg, throughout the duration of the experiments. The vitality parameters remained physiological during the first 8 hours of perfusion. For example, values measured in one perfused swine uterus are shown in FIG. 1 and FIG. 2.

#### 3.2. UTERINE CONTRACTILITY AND PERISTALSIS

Regularly recurring peristaltic waves, with IUP increases in both the corpus uteri and the isthmus uteri, were achieved in all of the perfused swine uteri and were continuously measured using the intrauterine double-chip catheter during the perfusion time. IUP changes showed similar values for the amplitude and duration of the uterine contractions during the whole duration of experiments.

One typical pressure profile is shown in FIG. 3. Data for increases in IUP are demonstrated as pooled data (each group contained 8 uteri) with mean values  $\pm$  SD in FIG. 4. A dose-dependent increase in IUP in the isthmus ( $P < 0.005$ ) and corpus uteri ( $P < 0.005$ ) was observed. The pressure increase was significantly higher in the isthmus uteri than in the corpus uteri at all concentrations tested resulting in a cervico-fundic pressure gradient. In addition, significant more peristalsis started in the isthmus uteri and moving in the direction of the corpus uteri ( $P < 0.001$ ) as shown in FIG. 5.

### 4. DISCUSSION

All physiological functions of the uterus may be influenced and modulated by uterine contractility e.g. sperm, oocyte and embryo transport and implantation and uterine contractility plays a key role in onset labour. Furthermore uterine contractility may be also influenced by endometriosis. Our aim was not only to establish an ex-vivo perfusion model of non-pregnant swine uteri to examine the effects of different test substances of uterine contractility but also to show directed peristalsis which

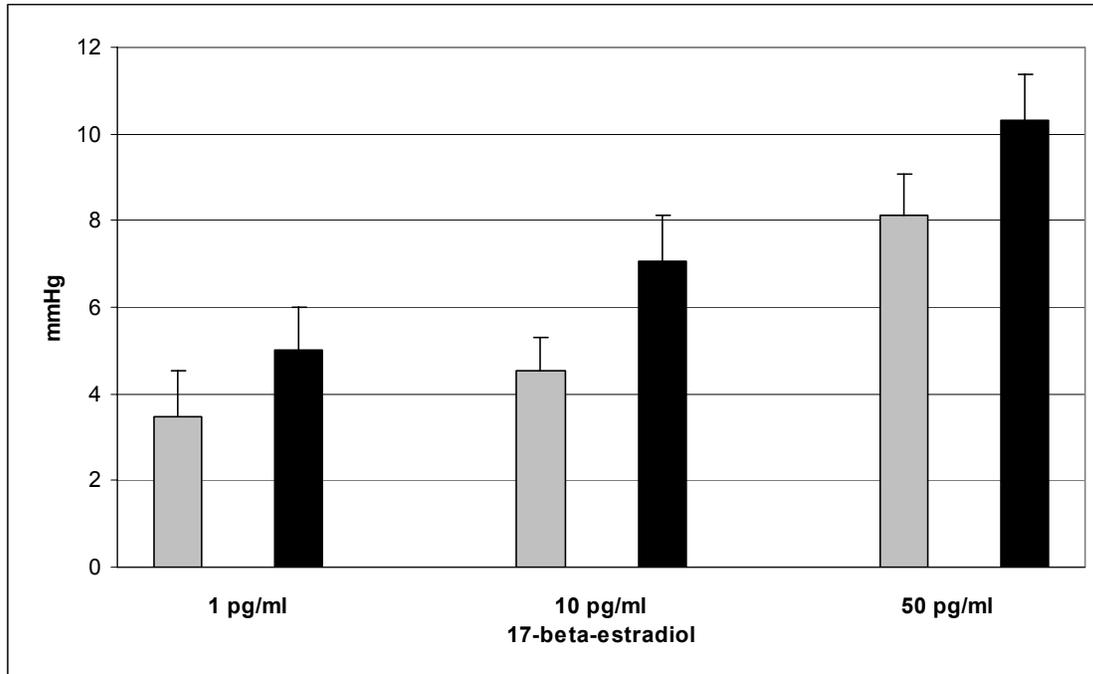


FIGURE 4. THE INCREASE IN INTRAUTERINE PRESSURE (IUP) DURING PERFUSION WITH DIFFERENT CONCENTRATIONS OF 17 $\beta$ -ESTRADIOL (GREY BARS, IUP MEASURED IN THE CORPUS UTERI; BLACK BARS, IUP MEASURED IN THE ISTHMUS UTERI; N = 8 UTERI EACH CONCENTRATION TESTED). A paired *t*-test was used to compare the increase in intrauterine pressure (IUP) in the isthmus uteri and in the corpus uteri. The pressure increase was significantly higher in the isthmus uteri than in the corpus uteri at all concentrations tested (*P* values: 1 pg/mL = 0.007, 10 pg/mL = 0.002, 50 pg/mL = 0.001). ANOVA: Estrogen perfusion resulted in a dose-dependent increase in IUP in the isthmus (*P* < 0.005) and corpus uteri (*P* < 0.005).

may be responsible for directed sperm transport mechanisms to the side bearing the dominant follicle. First we tried to use human uteri, but we have to face the problem – how to obtain an adequate number of physiologically intact uteri from young healthy women. Hysterectomy is performed due to pathological reasons and it is obvious that experiments with pathologically affected organs are hardly reproducible.

The advantage of the animal model used is that a large number of uteri equally in size and condition from young and healthy animals in their reproductive lifespan can be tested. Furthermore using uteri from young mammals makes it possible to detect definite differences in contraction peaks between the distal and proximal uterus compartments. The swine uterus may be more practicable than the human uterus for the *in vitro* study of uterine transport mechanisms caused by peristaltic contractions and waves, and their regulation, as the swine uterus

is more analogous to a muscular tube. The swine uterine cavity is more elongated than the human uterus. Peristaltic waves are easy to visualize, and intrauterine pressure changes at different locations can easily be recorded at the same time using an intrauterine multi-chip microcatheter. Delayed pressure changes may be better detectable in the swine uterus. In order to examine how long the uteri could survive in our perfusion system, some biochemical markers were assessed and demonstrated the feasibility of the swine uterus perfusion model at 37°C for a period of at least 8 hours, which offers the possibility of carrying out a large number of experiments with each uterus.

**ESTROGENS.** Visualization of uterine peristalsis using ultrasound and ultrafast magnetic resonance imaging showed different peristaltic patterns in the different cycle phases [4,8,25-27]. Richter et al. (2003) reported that oxytocin receptor expression is up-regulated by estrogens not only in pregnant

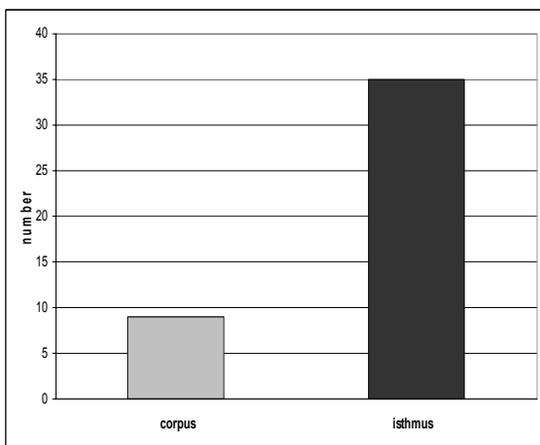


FIGURE 5. THE NUMBERS OF PERISTALTIC ( $N = \text{NUMBER} \times 10$ ) WAVES STARTING IN THE CORPUS UTERI (GREY BARS) AND ISTHMUS UTERI (BLACK BARS). Uterine contractions starting in the isthmus uteri or the corpus uteri were tested using the Chi-squared test of independence. There were significant more peristalsis starting in the isthmus uteri and moving in the direction of the corpus uteri ( $P < 0.001$ ).

human uteri but also in non-pregnant human uteri [15,16]. Stimulation with high dose  $17\beta$ -estradiol has been found to increase myometrial oxytocin receptor density, with maximum levels found in the uterine fundus [15]. Otherwise, long-term stimulation with oxytocin alone leads to mRNA down-regulation in human non-pregnant myometrial cells [16] and subsequently to an increase in myometrial activity only for the first time, followed by relative uterine quiescence during the further perfusion time [17]. It has therefore logically been suggested that estrogens may support uterine contractility and that they act synergistically with oxytocin in the regulation of uterine peristalsis and in the mechanism of transport towards the corpus uteri and the fallopian tubes. The results of previously published studies suggest that the effect of estrogens appears to be genomic via oxytocin receptor up-regulation and results in a significant increase in intra-uterine pressure increase [15,16,28]. The periovulatory cervicofundic peristalsis has been described as “rapid sperm transport” to the side bearing the dominant follicle, which is a precondition for successful reproduction in humans [3,4,8,9]. However, during menstruation, contraction waves appear to be directed toward the cervix [6,7]. Therefore an

estrogen-priming of the uteri was done with different estrogen concentrations to simulate hormonal environment during the periovulatory phase and to up-regulate oxytocin receptors as described by Richter et al. [15,16]. Furthermore the problem that the animals were in unknown stage of development in regards to the estrous cycle may be partly solved, using an estrogen containing perfusion buffer. The investigation of uterine contractility and its regulation by different hormones and mediators is of clinical interest not only in obstetrics – e.g., to induce labour or to delay birth and prolong pregnancy – but also in gynaecology and reproductive medicine e.g. in endometriosis and embryo implantation [29].

**OXYTOCIN.** In addition to steroids oxytocin is also believed to modulate myometrial contractility in a characteristic way, not only at the time of labour but also at the time of human reproduction [30]. Oxytocin levels fluctuate throughout the menstrual cycle and correlate with genital lubrication and sexual arousal in women [31-35]; they are therefore believed to play a role in the peripheral activation of sexual function [36-38]. Several studies have shown that levels of oxytocin increase during sexual stimulation and arousal, with a peak during orgasm in women and in men [31-35]. Oxytocin levels fluctuate throughout the menstrual cycle, correlate with genital lubrication in women, and are therefore believed to play a role in the peripheral activation of sexual function [36]. Suppression of endogenous oxytocin activity in women affects the ovulatory cycle. Evans et al. (2003) also demonstrated a biological correlation between oxytocin and the physiological processes of luteinizing hormone (LH) regulation in animal models [37]. Benoussaidh et al. recently reported that oxytocin modulates the activity of the uterus in a cycle-dependent fashion in rats [38]. Oxytocin increases during sexual arousal, and orgasm may therefore play a role in successful conception during coition, due to stimulation of uterine contractility and peristalsis. Oxytocin is used clinically to induce labour at term, as one of the most potent uterotonic agents, and exerts a wide spectrum of central and peripheral physiological effects [31-35,39-43]. Oxytocin produced by the posterior lobe of the pituitary

gland or locally in the uterus appears to be involved in the initiation of labour, while vasopressin is believed to play an important role in the uterine hyperactivity occurring in primary dysmenorrhoea [44-47].

In the present study we were able to show a cervico-fundal pressure gradient and an increasing number of peristaltic waves starting in the isthmus uteri and moving into the direction of the corpus uteri after estrogen-priming of the uteri and induction on peristalsis by oxytocin using the perfusion model described. Therefore this ex-vivo perfusion model can serve as an adequate model for studying uterine contractility, peristalsis and transport mechanisms in reproductive medicine and can be used for further in vitro experiments. Despite the general limitation of the results from animal models, which are not easily transferable to humans, this perfusion model may represent a useful and practicable method of studying the effects of different substances and hormones on uterine contractility and uterine transport mechanisms. Additionally, the bicornuate swine uterus is ideal for examining differences in side-dependent uterine transport mechanisms, since each horn can be perfused independently and pressure changes can also be measured in each horn independently.

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