The Role of Oestradiol in the Uterine Peristalsis in the Perfused Swine Uterus

T. Maltaris, R Dittrich, W Widjaja, C Sindhuwinata, I Hoffmann, MW Beckmann and A Mueller

Department of Obstetrics and Gynaecology, University-Hospital Erlangen, Erlangen, Germany

Contents

This study was designed to examine the effects of oestradiol (E2) on sperm transport in the swine uterus. The bicornuate swine uterus is optimal for the study of the uterine transport and peristalsis because the influence of various factors can be examined on each uterine horn independently. Forty swine uteri (with or without ovarectomy) were perfused for a period of up to 7 h. Two different E2 concentrations (3 or 30 pg/ml) in the perfusion medium were administered for 30 min unilaterally. Through an intracervical catheter 1 ml of a high concentrated dextran blue solution was administered directly in the upper part of the cervix. After bilateral perfusion of the swine uterus with a bolus of 0.3 IU oxytocin the distribution of coloured particles was assessed macroscopically before and after incision of the uterine horns. Coloration was evaluated by two observers blinded to the site-specific administration of E2. In the 10 ovarectomized uteri with the 3 pg/ml E2 concentration a unilateral distribution towards the side of oestradiol administration was observed in six uteri, in four it was a bilateral distribution. In the 10 non-ovarectomized uteri with the 3 pg/ml E2 concentration a unilateral coloration was observed in five uteri, in five it was a bilateral distribution. In the 20 uteri with 30 pg/ml E2, a unilateral coloration of the uterus horns was observed in all uteri. Oestradiol is one of the main factors, which influences the direction of the sperm transport in a dose-dependent manner, in the perfused swine uterus.

Introduction

In mammals oestradiol is the dominating hormone during the periovulatory phase when uterine contractions rise in frequency and amplitudes. In humans the oestradiol peak during the menstrual cycle occurs when an effective transport mechanism towards the fundus uterus and the Fallopian tube is needed for sperm transport (Lyons et al. 1991; Fukuda and Fukuda 1994). In the sow uterine contractility is maximized at the time around oestrus and can be increased after oestrogen administration (Langendijk et al. 2002a).

Perfusion models of various organs have been of great interest, particularly in the field of transplantation medicine and in studies of the physiology and metabolism of tissues. We have previously demonstrated that the perfusion model used here is capable of keeping the swine uterus in a functional condition for up to at least 7 h and that it is appropriate for the study of physiological questions (Dittrich et al. 2003; Maltaris et al. 2005a,b; Müller et al. 2006). The experimental system detects electrical and mechanical activities in the whole organ, as it maintains the architecture and intercellular relations of the uterus (Bulletti et al. 1988, 1993; Richter et al. 2000, 2003, 2004, 2006).

The swine uterus is a bicornuate organ and therefore ideally appropriate for examining the influence of various factors on the uterine transport and peristalsis because each uterine horn can be perfused independently. The swine produces many offspring in contrast to the humans and has no single dominant follicle. Therefore, under normal physiological conditions, there is no difference in oestradiol concentration in the uterine horns. The uterine lumen is a relatively hostile environment for the semen and uterine contractions are probably important for the transportation of sperm cells to the uterotubal junction as soon as possible after insemination (Langendijk et al. 2002b). There is few and sometimes contradictory literature available concerning the role of uterine contractility on the transport of sperm cells and fertilization (Baker et al. 1968; Rodriguez-Martinez et al. 1987a,b,c; Claus 1990; Langendijk et al. 2002b).

The present study examined the direct and isolated effect of two oestradiol concentrations regarding sperm transport, with or without ovarectomy in the perfused swine uterus. The aim of this study was to examine whether the increased oestradiol concentration in one uterine horn could stimulate the uterine contractility and thus direct the sperm transport towards this specific horn.

Methods

Swine uterus

Swine (Sus scrofa domesticus) are widely used in research. The female reproductive system in the swine has a bicornuate uterus with tortuous Fallopian tubes. The Fallopian tubes in an adult female have the same diameter as those in the human, but they are much longer. Forty swine uteri were obtained from a local slaughterhouse. They were selected on the basis of their size, overall condition and the condition of the uterine arterial stumps. The mean weight of the swine uterus was 149.8 g (105.6–230.2 g). They all came from healthy animals aged 5 months to 1.5 years. On the basis of previous observations involving perfusion experiments (Dittrich et al. 2003), it was decided that the ideal size of uteri for the experiments would be approximately 90–200 g. The swine uteri were very easily separated from the rest of the body in approximately 2 min shortly after the animal had been killed by electric shock (1.5 A, 400 V, 4 s).

T. Maltaris and R. Dittrich contributed equally to the study.
Perfusion system
After cannulation of both uterine arteries with 16- to 24-gauge needles (Abbocath-T; Abbott, Sligo, Ireland) depending on the size of the uterus, the organ was placed in a controlled-temperature perfusion chamber (Karl Lettenbauer, Erlangen, Germany) filled with the perfusion medium (Fig. 1a). The uterus was then connected bilaterally to 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 pumped into the uterine arterial catheters with two roller pumps. The flow rates of the perfusion medium and oxygenation were constantly monitored and kept at 15 ml/min and 0.05 bars, respectively, with an ideal pressure rate of 100 mmHg being maintained throughout the duration of the experiments.

17-β-oestradiol (3 or 30 pg/ml β-oestradiol, E4389; Sigma-Aldrich, Deisenhofen, Germany) was added in the perfusion medium and administered always only in one uterine horn. It is known (Almond and Dial 1990) that in the sow the maximum E2 serum concentration is at the periovulatory period (approx. 30 pg/ml), whereas the minimum E2 concentration is present at the diestrous (approx. 10 pg/ml).

Vitality parameters
Samples of the perfusate were taken at 1-h intervals after collecting the medium for measurements of pH, PO₂, PCO₂, HCO₃, lactate and oxygen saturation (for details, see Dittrich et al. 2003).

Study design
Twenty swine uteri were perfused after ovariectomy with two oestradiol concentrations (30 or 3 pg/ml) in order to simulate the hormonal environment of the sow in the oestrus and diestrus. In order to examine the effect of oestradiol on the sperm transport in the intact swine female genital tract, the experiment was also performed on twenty non-ovarectomized uteri.

Evaluation of blue dextran distribution
Thirty minutes after the continuous oestradiol administration was started, blue dextran solution (20 mg/ml Blue Dextran, D5751; Sigma-Aldrich) was administered. Blue dextran cannot be absorbed by the tissue and was used to simulate the semen fluid. Oxytocin (Syntocinon; Novartis Germany Ltd, Nuremberg, Germany) was used as a bolus through the uterine arterial catheters to induce contractions of the uterus at a dose of 0.3 IU. Uterine contractions occurred normally immediately after oxytocin administration.

Five minutes after blue dextran administration, two observers, blinded to the side in which oestradiol was administered, examined the uterus independently. Only if both examiners stated that the same uterus horn was blue coloured was the test ‘unilateral’, if one horn was not significantly more blue coloured, then the decision was ‘bilateral’. The uterus was then incised and photographed (Fig. 1b, c).

Results
In all 40 uteri there was a significant blue coloration of at least one of the uterine horns. In none of the 40 uteri could a contralateral blue coloration be detected to the oestradiol-treated uterine horn.

Group A (ovarectomized swine uteri)
In the 3 pg/ml group six uteri showed a significant ipsilateral coloration and four uteri showed a bilateral coloration (Table 1). In the 30 pg/ml oestradiol administration group all 10 uteri showed a significant ipsilateral blue coloration.

Group B (intact swine uteri)
In the 3 pg/ml group five uteri showed a significant ipsilateral blue coloration and five uteri showed bilateral

Fig. 1. (a) Perfused swine uterus in the organ bath. (b, c) Two swine uteri with unilateral blue coloration after incision
blue coloration. In the 30 pg/ml oestradiol administration group all 10 uteri showed a significant ipsilateral blue coloration.

**Discussion**

In humans adequate uterine contractility is involved in transport of the semen and gamete and in successful embryo implantation while inadequate uterine contractility may lead to ectopic pregnancies, miscarriages, retrograde bleeding and endometriosis (Leyendecker et al. 2004; Bulletti and de Ziegler 2005). Intact uterotubal transport function and directed sperm transport due to uterine contractility are therefore of critical importance in processes of human reproduction (Kissler et al. 2004, 2005). In fertile women the directed unilateral transport of immotile sperm-like material through the female genital tract to the side bearing the dominant follicle can be assessed using hysterosalpingoscintigraphy (Kunz et al. 1996, Kissler et al. 2004).

In swine, sperm cells have to be transported from the cervical end to the tubal end of the uterine horns after mating or artificial insemination. The length of the uterine horns is about 0.8 m during oestrus. Sperm transport is thought to be a combination of both passive and active transport (active sperm movement). The active sperm transport is thought to be responsible for the migration of sperm cells from the proximal uterus into the uterotubal junction and the oviduct. There a sperm reservoir is built, until about the time of ovulation, which acts as a barrier to sperm cells, because of its morphology, the consistency of the luminal mucus, oviductal motility, sperm-epithelial adhesion and ciliary movements of oviducal cells (Rodriguez-Martinez 2000). Passive transport is more important in the initial phase of transport from the side of the deposition to the proximal uterus and the uterotubal junction (Scott 2000). The passive transport is probably driven by the flow of intrauterine fluid-containing sperm cells, due to gravitational force, and uterine contractions (Baker et al. 1968; Scott 2000). This phase is also called rapid phase and lasts only some minutes (Rodriguez-Martinez et al. 2005). The transport of the spermatozoa to the site of fertilization constitutes the third phase and is regulated by the disappearance of the intraluminal mucus in the sperm reservoir, the increase in the flow of fluid by increasing ciliary activity towards the ampullary-isthmic junction, and/or the conspicuous motility of the myosalpinx (Rodriguez-Martinez et al. 1982; Rodriguez-Martinez 2000).

The bicornuate swine uterus is optimal for the study of the uterine transport and peristalsis because the influence of various factors can be examined on each uterine horn independently.

Swine have been increasingly used as biomedical research models during the last 20 years, as they are recognized as a suitable animal model for human disease on the basis of comparable anatomy and physiology. The use of uteri from freshly killed animals from the slaughterhouse had the logistical advantage that obtaining a large number of uteri every day only took a matter of minutes, and in addition, approval from an ethics committee or animal experimentation board was not needed, as no animals were killed for experimental purposes. Moreover, the uteri were derived from healthy young animals in their reproductive years.

Normally, the uterus would rapidly degenerate without constant perfusion. A complex perfusion system was therefore established that provided the uteri with a steady flow rate of circulation, a constant temperature and continuous oxygenation of the perfusion medium, simulating physiological conditions in every possible way. In contrast to previous swine uterus perfusion experiments the intrauterine pressure changes were not registered in order to avoid manipulation of the cervix by the intrauterine catheter which might be expected to affect the uterine activity or the migration of blue dextran solution. In half of the animals, ovariectomy was performed to avoid an influence in the hormone levels of the own ovaries. In the other half of the animals the ovaries were not removed, in order to examine the effect of uterine peristalsis in an intact swine uterus.

This study demonstrates that uterine contractility is mainly responsible for sperm transport from the cervical to the tubal end. Furthermore, we were able to demonstrate that oestradiol is a major factor determining sperm transport. As there were no other hormones, serum factors or seminal plasma factors in the perfusion system, it can be assumed that oestradiol was the only variable factor that influenced the transport of dextran blue solution towards the one uterine horn that received oestradiol treatment.

Our experiments showed that within 5 min the whole uterine horn was coloured with blue dextran solution. Other researchers have already found out that uterine contractility can promote the distribution of sperm-containing fluid throughout the whole genital tract, even enabling unilaterally, deep intrauterine deposited semen to be distributed over both horns (Rath 2000). The question remains as to how oestradiol which fluctuates characteristically during the menstrual cycle is involved in influencing uterine peristalis. Richter et al. (2003) reported that oxytocin receptor expression is upregulated by oestrogens not only in pregnant but also in non-pregnant human uteri (Richter et al. 2003, 2004). Franczak et al. (2002) also stated that estrogens are evident stimulators of uterine oxytocin receptors synthesis in gilts. Also an increase in intrauterine pressure in response to oxytocin was most evident in sows in estrous (Zerobin and Spörrl 1972; Langendijk et al. 2002a) and parturition (Zerobin and Spörrl 1972). Therefore, it can be logically suggested that oestrogens may support uterine contractility and act synergistically with oxytocin in the regulation of uterine peristalsis and transport mechanism towards the tubal ends.

To our knowledge this is the first study of unilateral administration of drugs by perfusion in an extracorpo-

---

**Table 1. Ipsilaterally blue coloured swine uteri after a 17β-oestradiol perfusion**

<table>
<thead>
<tr>
<th>Group</th>
<th>3 pg/ml 17β-oestradiol perfusion</th>
<th>30 pg/ml 17β-oestradiol perfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovariectomized swine uteri</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>(n = 20)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intact swine uteri</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>(n = 20)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
really perfused uterus. These experiments show that the perfused swine uterus is an appropriate system for examining the physiology of the genital tract and offers the possibility of examining the action of specific hormones or pharmaceuticals directly on one or both uterine horns. In these experiments the cervical-tubal sperm transport promoting effect of oestradiol was proved.

Acknowledgements
This work was supported by the University of Erlangen-Nuremberg ELAN funds.

References


Rath D, 2000: New strategies to minimize the number of sperm for pig. AI ESDAR Newsletter 5, 7 (abstract).


Submitted: 18.12.2005
Author’s address (for correspondence): Dr Ralf Dittrich, Department of Obstetrics and Gynecology, University of Erlangen-Nuremberg, Universitätsstrasse 21–23, D-91054 Erlangen, Germany. E-mail: ralf.dittrich@gyn.imed.uni-erlangen.de

© 2006 The Authors. Journal compilation © 2006 Blackwell Verlag