

The effect of progesterone and other hormones on the Fallopian tube ciliary beat frequency in mouse

Master of Science Thesis in the Master Degree Programme Biotechnology

KARIN LIND

Department of Chemical and Biological Engineering
CHALMERS UNIVERSITY OF TECHNOLOGY
Göteborg, Sweden, 2011

The effect of progesterone and other hormones on the Fallopian tube ciliary beat frequency in mouse
KARIN LIND

© KARIN LIND, 2011

Department of Chemical and Biological Engineering
Chalmers University of Technology
SE-412 96 Göteborg
Sweden

Supervisors: Anna Bylander, Joakim Larsson and Mattias Goksör
Examiner: Christer Larsson

This work was carried out for the Institute of Neuroscience and Physiology at Sahlgrenska Academy, University of Gothenburg in collaboration with the Department of Physics, University of Gothenburg.

Cover: Mouse of the type C57BL/6, the female reproductive tracts of the mouse and the structure of progesterone.

[Tryckeriets namn]
Göteborg, Sweden 2011

The effect of progesterone and other hormones on the Fallopian
tube ciliary beat frequency in mouse

KARIN LIND

Department of Chemical and Biological Engineering
CHALMERS UNIVERSITY OF TECHNOLOGY
Göteborg, Sweden 2011

The effect of progesterone and other hormones on the Fallopian tube ciliary beat frequency in mouse

KARIN LIND

Department of Chemical and Biological Engineering

Abstract

The inside of the fallopian tube is covered with epithelial cells. These cells are involved in the transportation of oocytes and sperms in the tube and are important for fertilization to occur. The hormone progesterone has been tested for its effect on the ciliary activity in the fallopian tube and previous studies have shown that progesterone reduces the ciliary beat frequency (CBF). There are (at least) two types of progesterone receptors present in the fallopian tube, the membrane progesterone receptors, mPRs and the nuclear progesterone receptor, nPR. The aim of this study was to examine which of these receptors that regulate the CBF. Different hormones, specific for a certain receptor or group of receptors, were used to measure the CBF in mouse *ex vivo*. The fallopian tube was dissected and chopped in small pieces for exposure of the cilia. Each tissue sample was investigated based on ciliary activity by an inverted bright field microscope and a high speed camera. After addition of a certain hormone the change in CBF was recorded during 60 minutes. Progesterone with a concentration of 30 nM gave an average decrease in CBF, -1.4 Hz ($p=0.03$). Promegestone R5020 is an agonist for nPR and gave a reduction in CBF, -3.13 Hz ($p=0.0046$) for the concentration 100 nM. An agonist for the mPR, substance X, was tested and gave no significant change in CBF. Mifepristone RU486 works as an antagonist for nPR and gave no significant change in CBF. The reduction in CBF by progesterone seen in this study is in agreement with that from previous studies. Reduction in ciliary activity by R5020 gives a clue to the involvement of nPR in the regulation of CBF in the fallopian tube in mouse. Based on the measurements performed in this study the mPR does not seem to be involved in the regulation of CBF.

Keywords: Fallopian tube, cilia, CBF, progesterone, mifepristone RU486, promegestone R5020, substance X

Abbreviations and glossary

CBF	ciliary beat frequency
DBD	DNA-binding domain
FSH	follicle stimulating hormone
IDA	inner dynei arms
LBD	ligand binding domain
LH	luteinizing hormone
mPR α , β , γ	membrane progesterone receptors alpha, beta, gamma
ODA	outer dynei arms
P ₄	progesterone
PBS	phosphate buffer saline
PCD	primary ciliary dyskinesia
PGR A/B	progesterone receptor protein A & B
PRL	prolactin
R5020	promegestone
RU486	mifepristone

Agonist

A substance that binds to a receptor of a cell and stimulates the activity of that cell.

Antagonist

A substance that upon binding to a receptor does not trigger any response, but blocks the effect from the agonist.

Ectopic pregnancy

A condition in which the pregnancy implants outside the uterine cavity.

Estrous cycle

Cycle of physiological changes in mammals with placenta that is induced by reproductive hormones.

Menstrual cycle

Cycle of physiological changes in female primates induced by reproductive hormones.

Populärvetenskaplig sammanfattning

Syftet med studien i denna rapport har varit att se hur progesteron påverkar rörelsen hos flimmerhåren i äggledaren hos mus och vilka mekanismer som är inblandade. Människan delar många gener med möss och reproduktionsorganen fungerar på liknande sätt varför mus är en bra modellorganism för att studera dessa effekter. Tidigare studier har visat att progesteron sänker rörelsefrekvensen hos flimmerhåren men man vet inte hur denna sänkning sker.

Det är oftast i äggledaren hos kvinnan som befruktningen sker. I väggen på äggledaren sitter det celler med flimmerhår. Dessa flimmerhår är viftande, hårliknande strukturer som hjälper till att transportera äggceller från äggstockarna till livmodern. När ägget släpps från äggstockarna fångas de upp av en trattliknande struktur som utgör en del av äggledaren. Därefter transporteras ägget mot livmodern samtidigt som spermier transporteras mot ägget med hjälp av flimmerhårens rörelser. Spermien befruktar ägget och tillsammans bildar de en ny cell, en zygot. Efter ca 5 dagar når sedan det befruktade ägget livmodern där det växer och blir till ett embryo som sedan utvecklas till ett foster. I vissa graviditeter utvecklas fostret i en av äggledarna istället för i livmodern och detta är ett tillstånd som kan leda till döden för kvinnan. Detta kallas utomkvedshavandeskap och har identifierats med ett antal riskfaktorer såsom infertilitet, rökning och olika typer av äggledaroperationer. Ett minskat antal flimmerhår har observerats i flera fall efter utomkvedshavandeskap vilket ger en indikation om att väl fungerande flimmerhår är viktigt för en lyckad graviditet på naturlig väg.

Det finns två typer av mottagarstrukturer, receptorer, för progesteron. En som är lokaliserad inne i kärnan och när progesteron binder till denna aktiveras gener som ger upphov till nya proteiner via en långsam respons. Nyligen har man även upptäckt en grupp av membranreceptorer som ger snabb respons då progesteron binder och ger upphov till en effekt i cellen utan att det behöver omfatta genaktivering. För att undersöka vilken av receptorerna som ger upphov till sänkningen testades olika hormoner som är mer eller mindre specifika för de olika receptorerna. Några av dessa ämnen binder till receptorn utan att ge någon egen effekt men hindrar då progesteron från att binda. Från detta kan sedan slutsats dras om via vilken receptor progesteron sannolikt utövar sin huvudsakliga effekt genom.

Table of Content

INTRODUCTION	1
Purpose	1
BACKGROUND	1
The Fallopian tube.....	1
<i>Secretory cells</i>	1
<i>Ciliated cells</i>	1
Menstrual cycle.....	4
The Estrous Cycle.....	5
P ₄ Progesterone.....	6
<i>Nuclear Progesterone Receptor</i>	6
<i>Membrane Progesterone Receptors</i>	7
Agonists and antagonists	7
<i>R5020 Promegestone</i>	8
<i>Substance X</i>	8
<i>RU486 Mifepristone</i>	8
MODELS.....	9
Mouse model	9
METHOD.....	10
Ethics	10
Tissue collection	10
Detection.....	10
Measurement	10
Calculations	12
<i>t-test</i>	13
RESULTS.....	13
<i>Ciliary Beat Frequency</i>	13
<i>P4 Progesterone</i>	13
<i>R5020</i>	17
<i>Substance X</i>	21
DISCUSSION.....	28
CONCLUSION.....	30
FUTURE WORK.....	30
ACKNOWLEDGEMENT	31
REFERENCES	32

INTRODUCTION

The fallopian tube is located between the ovary and the uterus in mammals. The inside of the tube is covered with ciliated epithelia. These cilia together with muscular contractions and the follicular fluid play a major role in the transport of sperms and oocytes and are important for fertilization [1]. Different hormones have been tested for their effects on ciliary activity. Progesterone has proven to cause reduced ciliary activity and may at a high level be a cause of ectopic pregnancy [2]. Ciliary dysfunction caused by endometriosis or smoking may be the reason for reduced fertility in some women [3, 4].

Purpose

Previous studies have shown that progesterone reduces the ciliary beat frequency, CBF, in mice with a fast response [5]. It is, however, not known which receptor or receptors that mediate this response. The attempt of this study is to use different substances that are specific for a certain receptor or receptor group. This will give a clue to if it is the membrane receptors or the nuclear receptor that is responsible for the response seen by progesterone. Then, by using knock-out mice missing the gene coding for the nuclear receptor, we aim to further establish which of the receptors that is responsible for the response. This thesis is part of a research project with the aim to provide knowledge about the possible effects of endocrine disrupting substances mimicking progesterone in humans and other mammals, as well as increasing our general knowledge of the physiology of the fallopian tube, i.e. establish a mechanistic understanding that eventually may be applied for example to reduce the risk for ectopic pregnancies.

BACKGROUND

The Fallopian tube

In mammals the Fallopian tubes are running between the ovaries and the uterus. It is in the Fallopian tube fertilization take place [5]. The Fallopian tubes of mice are curly compared to human fallopian tubes which are stretched out and measure about 10 cm in length. The fallopian tube and the ovary of the mouse are surrounded by a bursa which is lacking in humans [4]. The wall of the tube consists of three different layers, the internal mucosa, the outer serosa and in-between is the intermediate muscular layer. The internal mucosa of the tube is covered with a single cell epithelium layer. The epithelium consists of two different celltypes, secretory- and ciliated cells [6].

Secretory cells

Secretory cells are present in the fallopian tube and are non-ciliated epithelial cells. They help the capacitation of spermatozoa by secreting a fluid that contains nutrients. This fluid is also nourishing the embryo when moving down the tube. Invasion from microorganisms is also prevented by a fluid secreted by peg cells [7]. Peg cells are referred to as resting secretory cells. They are also non-ciliated and found in the fallopian tube and are only morphologically different from the secretory cells [8].

Ciliated cells

Cilia are present in large amounts in the Fallopian tube where they play an important role in the movement of ovary and sperms making fertilization possible. Each cilium is derived from a basal body which is developed from centriols. A cilium is on average 10 μm long and 25 μm in diameter. Inside the cilia there is a central bundle of microtubule known as

axoneme. The axoneme has two microtubules in the centre and these are surrounded by a ring of nine double microtubules. These cilia have the pattern 9+2 and are motile. Motile cilia are found on the tail of sperms and are of great importance for fertility *in vivo*. Movement of the axoneme is achieved by sliding of the double microtubules on one side of the axoneme while the other side remains passive. This movement requires the hydrolysis of ATP [3]. Polymerization of the microtubules is the fastest at the (+) end located at the tip of the cilia. The outer dynein arm (ODA) and inner dynein arm (IDA) are two structures attached to the microtubules and they are responsible for the sliding of the microtubules. This gives rise to a measurable controlled bending of the cilia called the ciliary beat frequency (CBF). CBF and the timing of a stroke cycle are mainly controlled by ODAs. The IDAs are responsible for the beat form and amplitude of the ciliary movement. The axoneme with the ODAs and IDAs is divided in two halves where one is responsible for the stroke generation and the other for the stroke recovery [9].

There are also cilia with the pattern 9+0 that are missing the two microtubules in the centre and are incapable of moving. Nonmotile cilia are found on epithelial cells in the kidney tubule as well as non epithelial cells such as neurons. They play a major role in signal transduction and chemical sensation [9].

The axoneme of the cilia is surrounded by a membrane. This membrane is an extension from the cell membrane where the cilia are attached. It is thought that nucleotides in the membrane control the CBF in the fallopian tube since this has proven to be the case in the epithelial cells of the airways. Studies done on the motile cilia of the human oviduct have shown presence of ion channels and polycysteins proteins in the membrane. These proteins are thought to be involved in facilitating the transport of oocyte and fertilization

since their level in the membrane is increased by the time of ovulation [9].

The proteins localized in the ciliary membrane are kept from entering the Fallopian tube membrane by a barrier structure known as the ciliary necklace. The cilia and the ciliary necklace can be seen in *Figure 1*. This cup-like structure is found at the base on both motile and non-motile cilia and is built up by intramembrane particles. Primary ciliary dyskinesia (PCD) is a genetic disorder that causes immotility or disordinated movement of the cilia. The condition is caused by mutations in ciliary proteins and can be the reason for male infertility. Lacking of ODAs is the most common reason for PCD [9]. The mentioned correlation between CBF and ODAs is supported in a study. The CBF of patients with PCD was measured and compared to that of healthy volunteers and the PCD patients had significantly lower CBF compared to the control group [10]. Ciliary necklace have been proposed to function as a timing device for ciliary beat. In a study performed on patients with PCD the necklace structure proved to be organised the same as in healthy persons. Despite this, it is likely that the necklace proteins are involved in the pathophysiology of PCD [11].

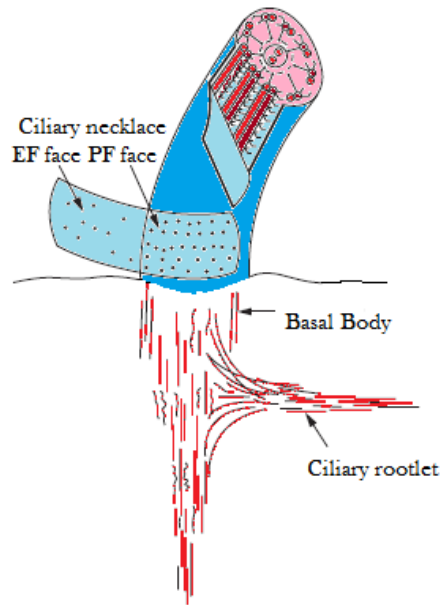


Figure 1: Illustrates a cilium with the ciliary necklace at the base (adapted from [11]).

The Fallopian tube consists of three regions in the following order from ovary to uterus; *infundibulum*, *ampulla* and *isthmus*. In Figure 2 the Fallopian tube and the distribution of the three regions can be seen. Ciliated cells are more abundant than secretory in the highly folded ampulla region. The isthmus region is only partly folded and rich in secretory cells [12].

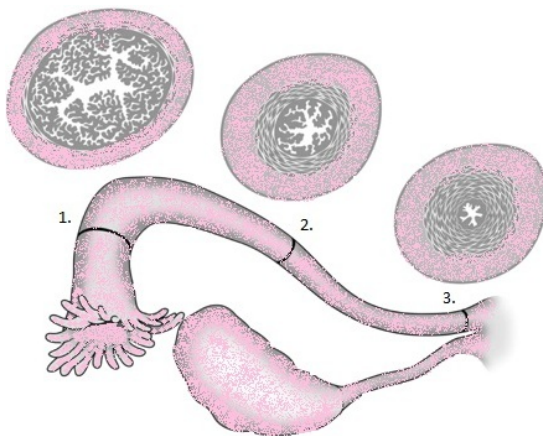


Figure 2: Illustrates the human Fallopian tube with cross-sections of the different regions 1) infundibulum 2) ampulla and 3) isthmus (adapted from [3]).

When an ovum has matured it is released from the ovary and caught by the *fimbriated* part of the *infundibulum* in humans. In mice, the ovum is released into the bursal cavity and travel to the fallopian tube by going through the bursal fluid [4]. The egg is then transported down the Fallopian tube by the flow of follicular fluid [3]. In mice several ova are transported close to each other [4]. The egg stays in the *ampulla* region for about 80 hours allowing fertilization to occur. After intercourse the sperm appears to bind to the epithelium at the isthmus region of the tube. The sperms then mature and release their binding and are transported up the tube by ciliary activity. It is in the *ampulla* region that the egg and sperm meet. The egg, after successful fertilization called a zygote, is further transported to the uterus by cilia activity and contraction of the tube muscles [3]. Studies have shown that if blocking the muscles in the tube the ovum is still transported to the uterus in a normal time frame. This gives evidence to the major contribution of ciliary activity for a successful fertilization and embryogenesis. The hypothesis is further supported by the increase in CBF after ovulation [13]. However, some women suffering from a syndrome where the cilia are defected have been shown to be fertile. This might indicate that ciliary activity, muscle contraction and hydraulic pressure all are involved in the events leading to a successful fertilization [14].

CBF is known to be affected by ovarian steroids in different ways. Estrogen increases the production of cilia on the ciliated cells of the Fallopian tube epithelium and increases the production of tubular fluid [13]. Progesterone affects the mucosa of the Fallopian tube by decreasing CBF and there is evidence that high progesterone levels are related to the occurrence of ectopic pregnancies [15]. When testing the effect of progesterone on the Fallopian tube cilia a reduction in CBF could be seen after only 30 minutes. This rapid response suggests that progesterone

regulates the CBF in a non-genomic hormonal interaction. Fallopian tubes of both mouse and cow have given this fast response suggesting this may be true for even more mammals [5].

The mucosa layer of the fallopian tube is thick and gives an adequate environment for fertilization to occur in the *ampulla* region. In the *infundibulum* the mucosa is involved in the movement of the ovum from ovary to uterus with the help of a supportive fluid [4].

Menstrual cycle

The general outline of the reproductive system is shared between all mammals but when seeing to the function in more detail there are some variations. Human females go through the menstrual cycle which is a cycle of physiological changes involving many hormones [16]. The mouse and other non-primates go through the estrous cycle that is similar to the menstrual cycle [17].

The menstrual cycle is divided into three phases, the follicle phase, ovulation and the luteal phase. The menstrual cycle can be seen in *Figure 3*. During the follicular phase the amount of estrogen increases and there is a thickening of the uterus lining. The lining contains nutrients and fluids for the embryo. Several hormones are

involved in the development of follicles in the ovary. Estrogen is synthesized and secreted by the influence of follicle-stimulating hormone (FSH). This development proceeds for a few days and at the end only one or two follicles remain while the others have died. The follicle phase ends with an increase in luteinizing hormone (LH). In the ovulation phase the remaining follicle releases an egg due to the high levels of LH. The LH and FSH decreases during the last phase of the menstrual cycle. The follicle is now turned into a *corpus luteum* which will produce the progesterone that is necessary for fertilization of the egg. The egg lives for 24 hours and has to be fertilized within this time. In the presence of progesterone and estrogen the lining of the uterus will change to prepare for the implantation of an embryo. Progesterone also hinders sperm to enter the uterus and also help keep bacteria away. In the absence of implantation the *corpus luteum* will degenerate and a new menstrual cycle will begin. If fertilization occurs a hormone called human chorionic gonadotropin will be produced that sustain the *corpus luteum*. The fetus will eventually start producing its own hormones but until then progesterone will be provided by the *corpus luteum* [16].

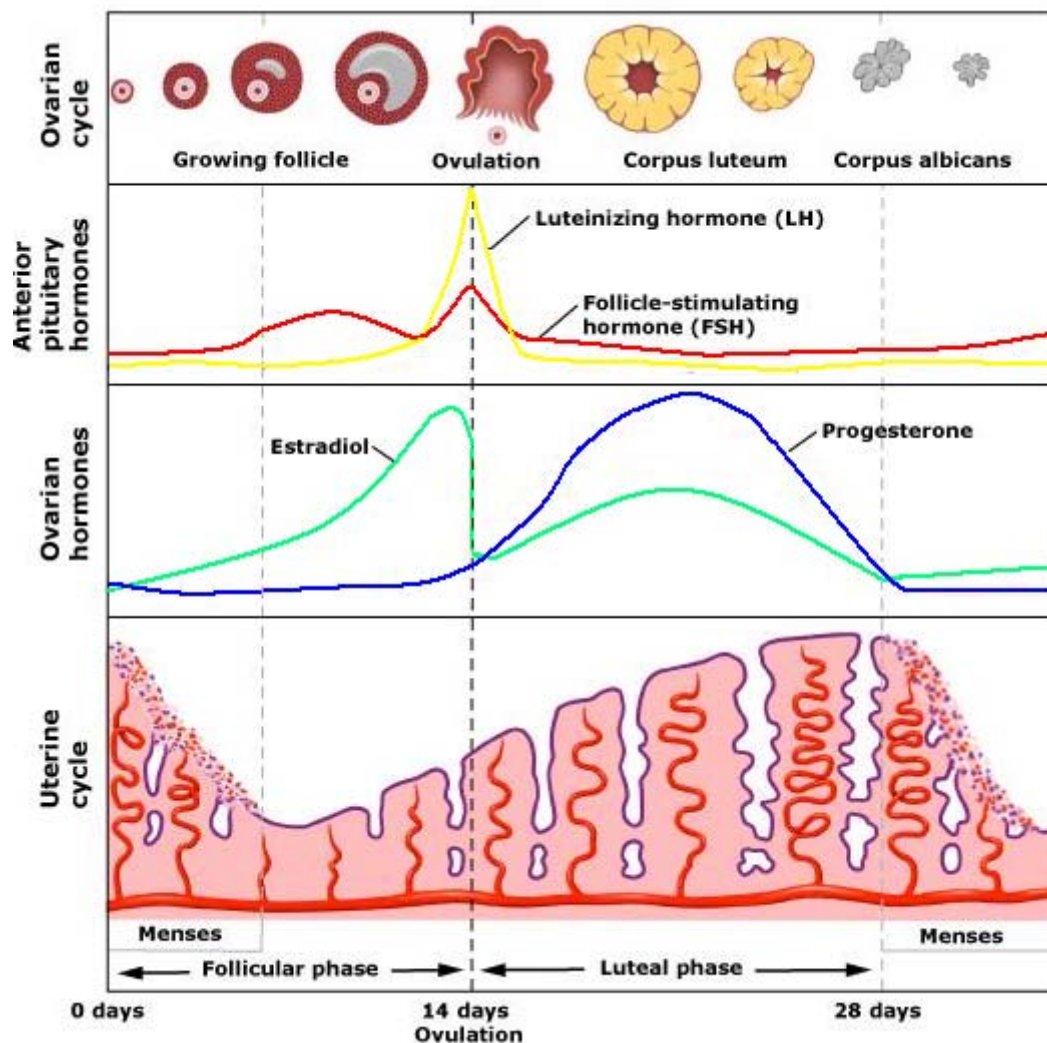


Figure 3: Illustrates the menstrual cycle in the female (adapted from [18]).

The Estrous Cycle

The estrous cycle is initiated after puberty in almost all female mammals with a placenta. In mouse the average estrous cycle is 4-6 days and, as for all mammals, it is divided in four phases, diestrus, proestrus, estrus and metestrus. To more resemble the menstrual cycle it can be said to consist of two phases, follicular phase and luteal phase [19].

In the proestrus phase the follicles in the ovary starts to grow and reach maturity. The endometrium of the uterus starts to develop when estrogen is produced. Ovulation of the mature oocytes occurs in

the estrus phase. This is done under the regulation of gonadotropic hormones. The phases are the same for all mammals but the number of eggs that ovulate is individual for each species. About 15 eggs ovulate simultaneously in the female mouse. The estrus phase is the only time during the cycle when the female mammal is sexually active. This can be compared to the menstrual cycle where the female can be sexually active throughout the entire cycle. During the metestrus the eggs move through the oviducts to the uterus. *Corpus luteum* starts to form and in some cases the animal may be bleeding due to low estrogen levels. Progesterone is produced

by the uterine lining during this phase which last for 1 to 5 days. The diestrus phase is the end of the previous ovulation and small follicles are secreted in the absence of pregnancy. The endometrium is reabsorbed and this is different from the menstrual cycle where the endometrium is shed. For rodents, such as mouse and rats, a plug will be formed in the entrance of the vagina. This plug is formed by components of the male ejaculate [19, 20].

P₄ Progesterone

Progesterone (P₄) belongs to the steroid hormone class progestogens. They are synthesized from cholesterol in several reaction steps catalyzed by enzymes. The role of progesterone in the body involves many reproductive functions such as maintenance of pregnancy, release of mature oocyte and facilitation of implantation [21]. Before ovulation progesterone is produced by the adrenal glands and after ovulation the site of production is the *corpus luteum* [4]. The release of progesterone is mainly simulated by luteinizing hormone (LH), another hormone involved is FSH. Other regulators are prostaglandins, β -adrenergic agents and prolactin, PRL. When the progesterone has been released it is bound to transcortin and transported in the blood [22].

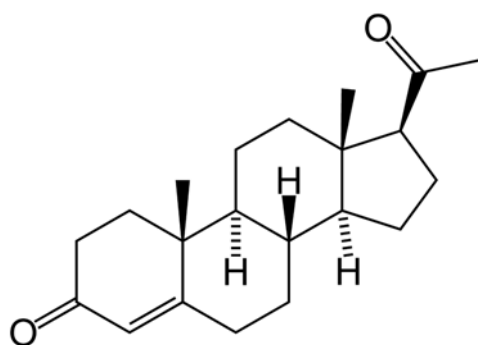


Figure 4: Structure of progesterone (P₄) [23].

Effects of progesterone can be genomic leading to an altered gene transcription upon activation of the nuclear progesterone receptor. More rapid, non-genomic effects have also been seen in tissues lacking this

nuclear PR leading to the discovery of a progestin membrane receptor [24].

Nuclear Progesterone Receptor

Nuclear receptors constitute a superfamily in eukaryotes involved in variety of processes including development and reproduction. This superfamily can be further divided in three classes; the steroid receptor family, the thyroid/retinoid family and the orphan receptor family. The estrogen receptor (ER) and the progesterone receptor (PR) belong to the steroid receptor family. Each nuclear receptor contains a C-terminal (the end of the amino acid chain characterized by a free carboxyl group) ligand-binding domain (LBD) and a DNA-binding domain (DBD). These domains are connected via an amino acid sequence. The LBD contains a binding site for the ligand or hormone and is responsible for recruiting coactivating proteins via a transcriptional activation function. The DBD transmit information between the different regions of the receptor. When the ligand binds to the receptor it releases heat shock proteins that bind to promoter sites upstream and that functions as maintenance of steroid receptors and transcription factors [25].

The progesterone receptor is expressed in many tissues including the uterus, ovary and breast in the female. It is also expressed in the brain and lung in man and woman and in the testes in men [22]. The progesterone receptor (PR) exists in two isoforms, PR-A and PR-B. These two forms are almost identical with the exception of 104 amino acids lacking in the N-terminus of the A-receptor [4]. Also, PR-B is a stronger transcriptional activator of target genes compared to PR-A but PR-A can suppress the activity of PR-B [22]. The response to progesterone by a cell is determined by the expression of the progesterone receptor as well as by the ratio of PGR-A to PGR-B [4]. In most of the tissues PR-A and PR-B are coexpressed but the regulation of the proteins are tissue specific. Shao *et al.* [26]

performed a study where the fallopian tube and uteri from mouse first was exposed to progesterone and then treated with a PR antagonist, RU486 *in vivo*. The treatment gave an increase in expression of PR protein isoforms [26]. Studies of the rat uterus *in vivo* showed that increasing levels of estrogen resulted in induced progesterone receptors protein and mRNA [27]. Early in the reproduction stage PR protein isoforms are thus most likely important for the tissues responsiveness to estrogens. It is also suggested that increasing levels of progesterone inhibit the expression of PR proteins [26].

PR has been shown to mediate its response in two ways. One is by the already mentioned genomic effect involving gene transcription and translation inside of the nucleus. PR also mediates more rapid, non-genomic response by activating a cell-signaling pathway outside of the nucleus. The amino-terminal domain of PR contains a motif that is rich in proline and function as a recognition site for SRC Homology 3 domain (SH3 domain). SH3 domain is a protein consisting of about 50 amino-acids residues that function by mediating the binding of PR to different signaling molecules in the cytoplasm [28]. PR-B has been shown to shuttle between the nucleus and the cytosol and mediating these rapid responses while PR-A has been shown to remain in the nucleus [29].

Membrane Progesterone Receptors

In 2003 the first study suggesting a membrane receptor for progesterone in fish was published [30]. Three different types of membrane receptors for progesterone (mPR), α , β , γ have then been identified in humans and mice [31]. The three types of mPRs are found in different kind of tissues. mPR γ has been found in the fallopian tube and ovary of the female mouse and also in the fallopian tube of humans where it has been associated with the membrane at the base of ciliated cells [24]. mPR β has also been found in ciliated cells and these progesterone membrane

receptors are likely to play a part in ciliary activity and transportation of gametes. The location of mPR in the fallopian tube is similar in mice and humans. mPR β is found in large amount in the ovary, uterus and the fallopian tube in the female and in the testis of males [24, 32]. mPR α have been found on the membrane of sperms in some fish species. The localization of the membrane receptor is on the region of the sperm that is involved in the motility. It is therefore believed that sperm hypermotility is regulated by stimulation of this receptor. In mouse and human mPR α mRNA has been expressed in the testis. In a certain fish species mPR α has been found in different tissues such as brain, reproductive, heart and intestine. This distribution is very similar to that of the nuclear progesterone receptor [33]. In human myometrial cells progesterone and estrogen have been shown to upregulate mPR α . mPR β was significantly up-regulated by estrogen alone [10]. It has also been demonstrated that mPRs are present in the follicles of the ovary in mammals and it is suggested to be involved in apoptotic processes [34].

Agonists and antagonists

In this study synthetic substances have been used as either agonist or antagonist to the progesterone receptors. The agonists bind to the receptor and trigger a response. In this study the agonists used are R5020 and substance X and they are both designed to mimic the action of progesterone. When an antagonist binds to a receptor it does not trigger any response of its own (or is a weak trigger) but primarily blocks the effect of the agonist if this is present [35]. In this study RU486 is used as an antagonist for progesterone.

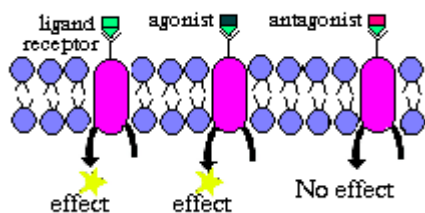


Figure 5: Shows the response when a ligand, agonist and antagonist, respectively bind to a receptor (adapted from [36]).

R5020 Promegestone

R5020 is a synthetic progestin and a progesterone agonist. It is a selective agonist for the nuclear progesterone receptor and has low affinity for the progesterone membrane receptor [34]. Compared to progesterone it has a higher affinity for the progesterone receptor and dissociate slower when bound. Studies have shown that R5020 bind with $K_a = 8.8 \times 10^8$ 1/mol to the progesterone receptor in rat uterine cytosol compared to the binding of progesterone $K_a = 1 \times 10^8 - 1.7 \times 10^8$ 1/mol [37]. Low concentrations of ATP have been shown to enhance the binding of R5020 to the progesterone receptor. Experiments have shown that R5020 most certainly bind to the receptor at the same site as progesterone [38].

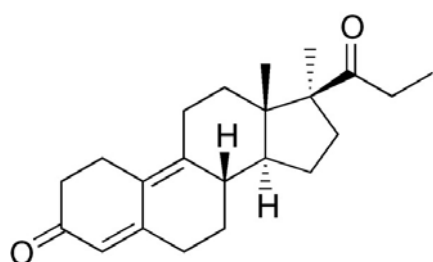


Figure 6: Structure of Promegestone (R5020) [39].

Substance X

Substance X is an experimental candidate drug designed to have strong affinity for the progesterone membrane receptor and working as an agonist.

RU486 Mifepristone

RU486 is an artificial steroid that works as a progesterone nuclear receptor antagonist in the presence of progesterone. When progesterone is absent mifepristone acts as a partial agonist. It has proven to have a number of clinical applications. RU486 is used as an abortifacient and has contraceptive action. It is also being used for the treatment of endometriosis and by aiding cervical dilation [40]. Mifepristone inhibits the function of follicle development and ovulation if given during the first half of the cycle. In high doses (100 mg) mifepristone has also proven to inhibit the implantation and function of *corpus luteum* [41]. Studies have shown that RU486 binds to the progesterone receptor with higher affinity than progesterone. The binding of RU486 to the receptor gives an irreversible effect. When binding, RU486 seem to transform the receptor it binds to [38]. Most data suggest that progesterone and mifepristone bind to the receptor at the same site. However, an experiment performed with labeled progesterone and RU486 indicated separate binding sites for antagonist and agonist to the receptor in the oviduct of a chicken [40]. RU486 acts as a pure antagonist towards PGR-A and as a partial agonist towards the PGR-B [25].

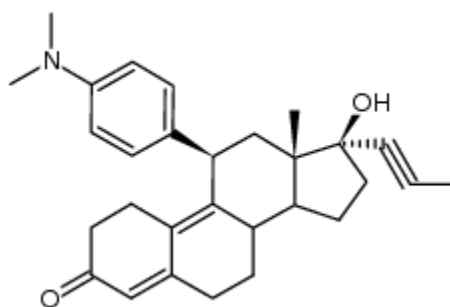


Figure 7: Structure of Mifepristone (RU486) [42].

MODELS

Mouse model

Mice are widely used as models for research on mammalian physiology and human diseases. This is in part because the mouse whole genome is known and about 85% of this genome is shared with human [43]. Using mouse for testing the effect on CBF by different substances has many advantages. The mouse is small, easy to handle, has a short life-span and a rapid reproductive rate [44]. Even though the female reproductive organ doesn't look exactly the same in human and mice on a macroscopic scale (see *Fig 8*) their general functions are similar [4]. What has to be considered is the fact that a mouse is not human and thus all results obtained from the animal studies cannot be directly transferred to humans.

All experiments were carried out *ex vivo* even though this can give slightly different result compared to *in vivo*. Animal trials were carried out on mice and the human aspect will only be discussed based on literature and previous studies.

The first set of the experiments were performed on the mouse strain C57BL/6. This strain is widely used for its good temper, robustness and the fact that they are easy to breed. On average the matings are productive in 84% of the cases and the litter size is 7 with an average of 4 litters [44]. Female mice reach sexual maturity in 6 weeks but can mate from 5 weeks. When reaching sexual maturity they start to produce progesterone that affect the CBF and thus all experiments were performed on mice 3-5 weeks of age.

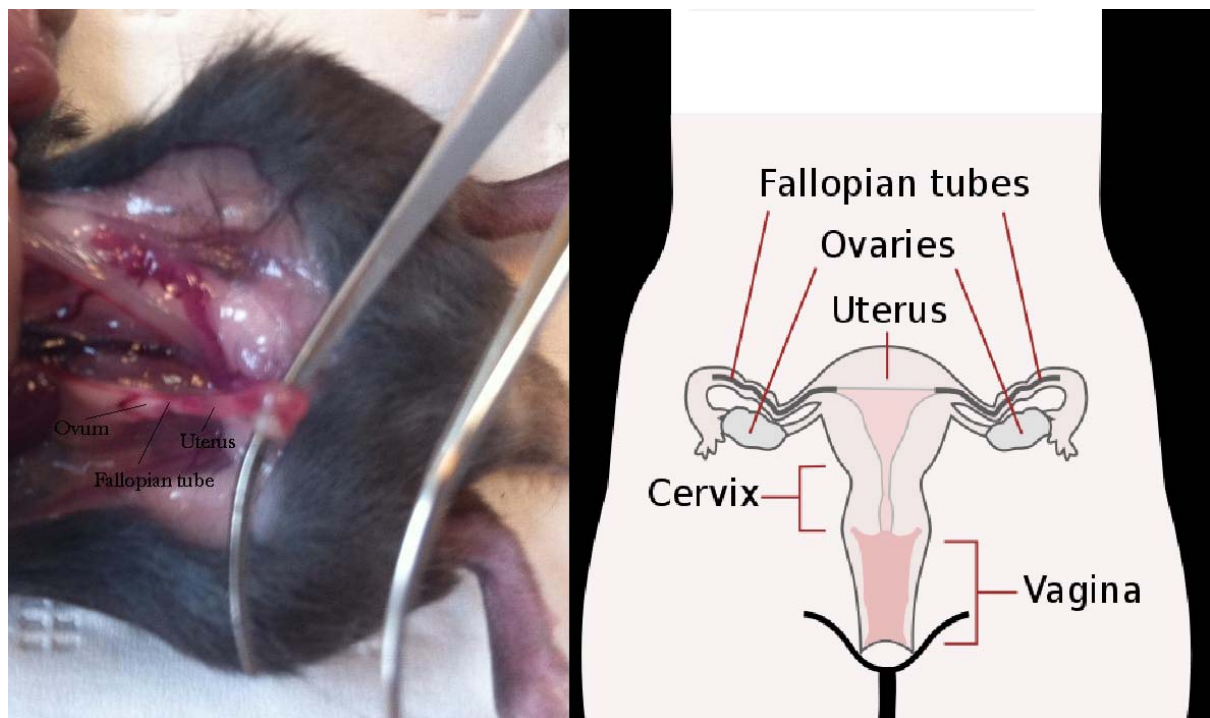


Figure 8: Anatomical structure of the reproductive system of the female mice (left) and human (right) [45].

METHOD

Ethics

The experiments performed during this study have been approved by the local animal ethics committee at Gothenburg University, Gothenburg Sweden (50-2011) to DGJ Larsson.

Tissue collection

Immature female mice C57BL/6 were obtained from Charles River, Kisslegg, Germany. The arriving mice were about 3 weeks old and allowed to acclimatise for at least 5 days before initiation of experiments. During the acclimatisation the mice were kept at 21 ± 2 °C with unrestricted access to food and water and a light cycle of 10 hours. The mice were put in a plastic box and a lid with a tube leading to a carbondioxide tank was put on top. A tap with carbondioxide at the concentration of 5 L/min were turned on and the mice were killed by suffocation.

To make sure complete death the neck was dislocated. Each mouse was dissected and the fallopian tubes together with the uterus were taken out. The sample was put in a petri dish containing PBS (phosphate buffer saline, pH 7) for rinsing. When the fallopian tube was cleaned from fat and connective tissue it was further cleaned in a new petri dish containing fresh PBS. It has been shown that the *infundibulum* and *ampulla* region of the fallopian tube contains the majority of the ciliated cells so these regions were cut out and put in a petri dish with the serum-free nutrition medium F10 with 10% fetal bovine serum and 1% ampicillin [4]. The tube segments were chopped in small pieces and opened longitudinally for exposure of the cilia. Measurement of CBF was carried out the next day and the samples together with the media were put in an incubator over night (37 °C, 5% CO₂).

Detection

An inverted bright field microscope (Nikon TE-300, Nikon Instruments. Inc, New York) was used when measuring the ciliary beat frequency. The objective (100x oil immersion) was kept at a constant temperature (36.5 °C) by an adjustable heater regulated by a controller unit (Biopetechs, Butler, Pennsylvania). The movement of the cilia was recorded by a high speed camera (Prosilica EC1020, Prosilica Inc, Burnaby, Canada) with the speed of 100 frames per second. The cilia are measured in an area of interest (50x50 pixels) and the change in light intensity caused by the ciliary movement is recorded by the camera. An example of fallopian tube pieces with beating cilia as shown on the screen can be seen in *Figure 9*. In LabVIEW a center-of-mass algorithm was used to convert this change in light intensity to a beating frequency by a fast Fourier transformation. The frequency of the cilia (in Hertz) in vertical and horizontal component was shown on the screen with the amplitude on the y-axis. An example how the frequency of the beating cilia is visualized can be seen in *Figure 10*.

Measurement

1 mL of G-MOPS was added to a petri dish and that was placed under the microscope. Pieces of the fallopian tube were added to the dish and the same amount of liquid added was removed to keep the volume in the petri dish constant. Once beating cilia was located the sample was fastened with a fine glass needle controlled by a joystick. The baseline CBF was measured 7 times during approximately 10 minutes. 500 µl of G-MOPS was removed and 500 µl of the substance of interest was added to the petri dish. CBF was then measured every 5 minutes for an hour. The substances had been dissolved in ethanol (70%) and then diluted with G-MOPS Plus-medium to reach the final concentration with an ethanol concentration of 0.02%. Each substance tested along with their

concentrations can be seen in *Table 1*. For each measuring session the CBF was measured on a control cell exposed only to ethanol (final concentration of 0.02%). It should be noted that when using mifepristone as a substance the sample was incubated with mifepristone for 30 minutes

before initiating the measurements. The initial volume in the petri dish was 2 mL. 1 mL G-MOPS was then removed and replaced with 1 mL RU486.

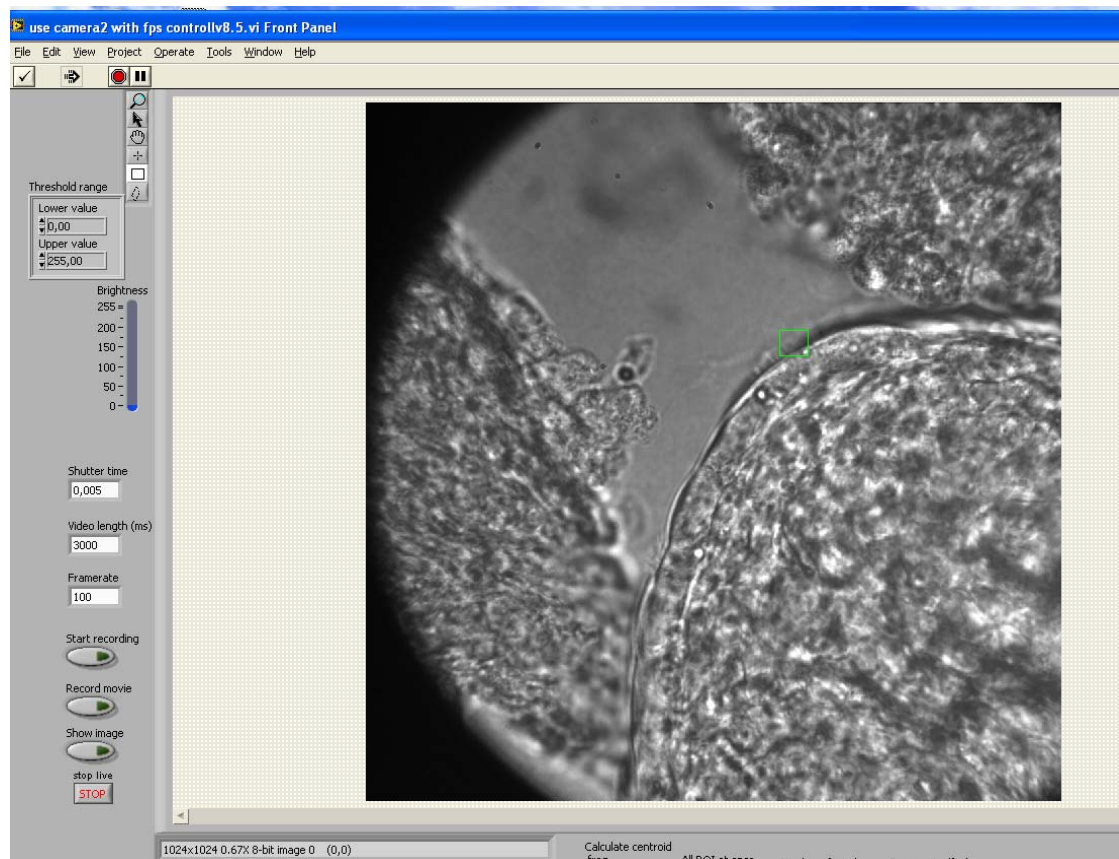


Figure 9: Fallopian tube pieces with beating cilia. The green box indicates the area of interest that is recorded.

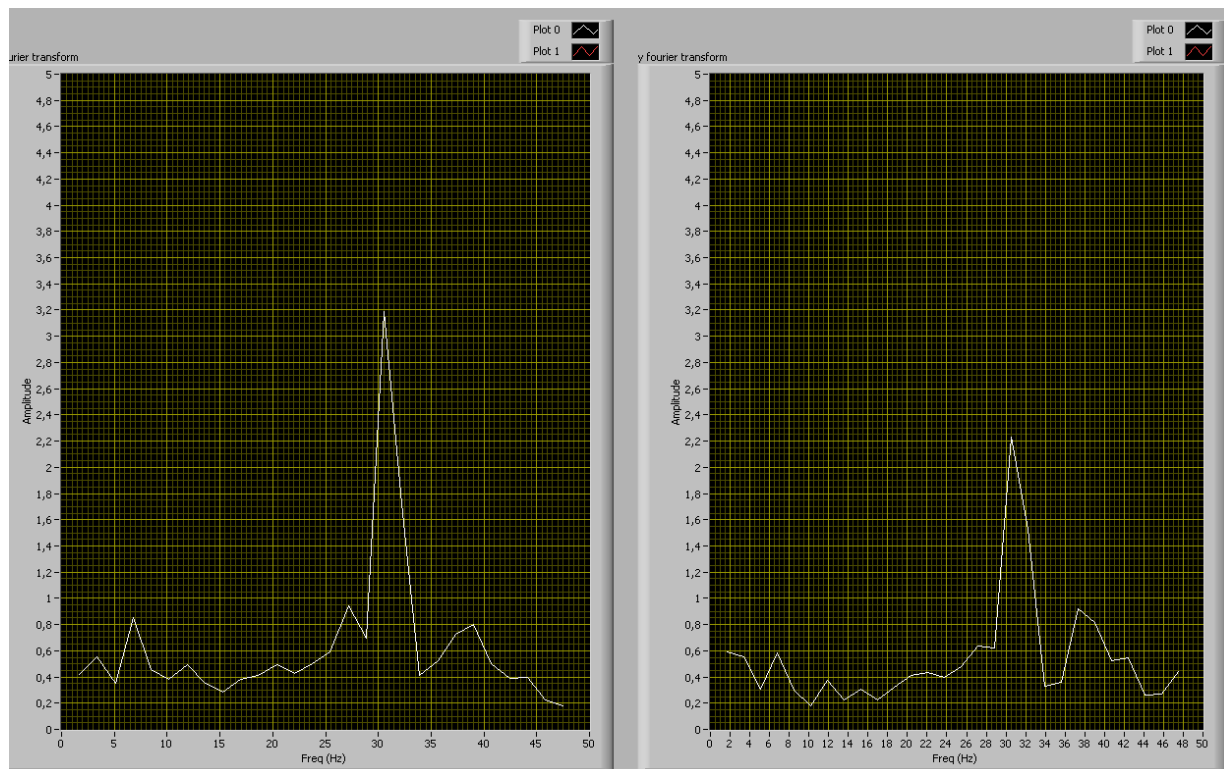


Figure 10: Frequencies found for the beating of the cilia in the fallopian tube. As can be seen some of the regions of frequencies are more pronounced than others. Note that the y-axis is in logarithmic scale.

Table 1: Shows the substances tested together with the concentrations.

P₄	X	R5020	RU486
10 nM	10 nM	10 nM	1 μ M
30 nM	100 nM	100 nM	
100 nM	500 nM	500 nM	
	2.5 μ M		

Calculations

The CBF analysis was based on measurements of cells that have been exposed to the substance of interest and cells exposed to ethanol only. From each mouse, we always included both control cells and cells treated with hormones. When the estimate of CBF was based on two cells measured simultaneously on the same tissue sample, the average of these was calculated first. Each measuring point was then normalized by subtracting it with the average baseline CBF value (average of first 10 minutes) for that cell. This was done both for control cells over the entire analyses period and for cells treated with hormones. Each normalized CBF value

was then subtracted with the normalized CBF value for the control cells from the same individual mouse.

An average for the CBFs between 30 min and 1 h after addition of a substance was calculated and the average for the 7 measurements initial to addition of this substance was subtracted with that CBF. This was also done for the control cells. When estimating a possible effect of the added substance a one-sided, unpaired student's t-test was used with a significance level of 0.05 comparing the treated cells with the control cells. A one-sided test was motivated by the previous knowledge of the effect of progesterone in

this system (decrease of frequency), and the hypothesis that the agonists mimic the effect of progesterone and antagonist cause the opposite effect.

For every substance and all concentrations a mean value CBF was calculated and a box plot was constructed using OriginPro 8 and CorelDRAW X4. A Standard error mean was calculated for each mean value and visualized in the box plot.

A graph containing the normalized average CBF and standard deviation at each measuring point for every substance and concentration was constructed using Excel and CorelDRAW X4.

t-test

The calculated probability value shows the probability of getting the calculated t value by chance alone. A low probability value gives a higher chance of the mean of the substance and the mean of the control being significantly different.

RESULTS

Ciliary Beat Frequency

The CBF for the cells during the baseline measurements were 29.2 ± 6.2 Hz. This is based on 151 measurements where the CBF range from 13 to 44 Hz. Based on the measurements for the control cells there was an average increase in CBF, 0.47 Hz when comparing CBF before and after treatment.

P4 Progesterone

The progesterone concentrations tested were 10 nM, 30 nM and 100 nM. For each concentration the average change in CBF, when comparing mean CBF prior to treatment with the mean CBF between 30-60 minutes after addition of progesterone, was calculated. The average CBF and standard deviation for each concentration and control cells, exposed only to ethanol, is visualized in *Figure 11*. For progesterone 30 nM there was an significance average decrease in CBF, -1.4 Hz ($p=0.03$). The progesterone concentration 10nM gave an decrease in average CBF, -1.22 Hz ($p=0.09$) and 100nM gave an average decrease of -1.81 Hz ($p=0.06$).

CBF was measured every 5 minute for 60 minutes after addition of P_4 and normalized to the mean CBF prior to treatment. The CBF values were then normalized to the control cells (treated with ethanol). In *Figure 12* the normalized change in CBF prior to any treatment and after addition of P_4 10 nM is plotted. In *Figure 13* and *Figure 14* the normalized change in CBF after addition of P_4 30 nM and P_4 100 nM respectively, are plotted. A decrease in CBF can be seen after hormone treatment with progesterone 30 nM *Figure 13*.

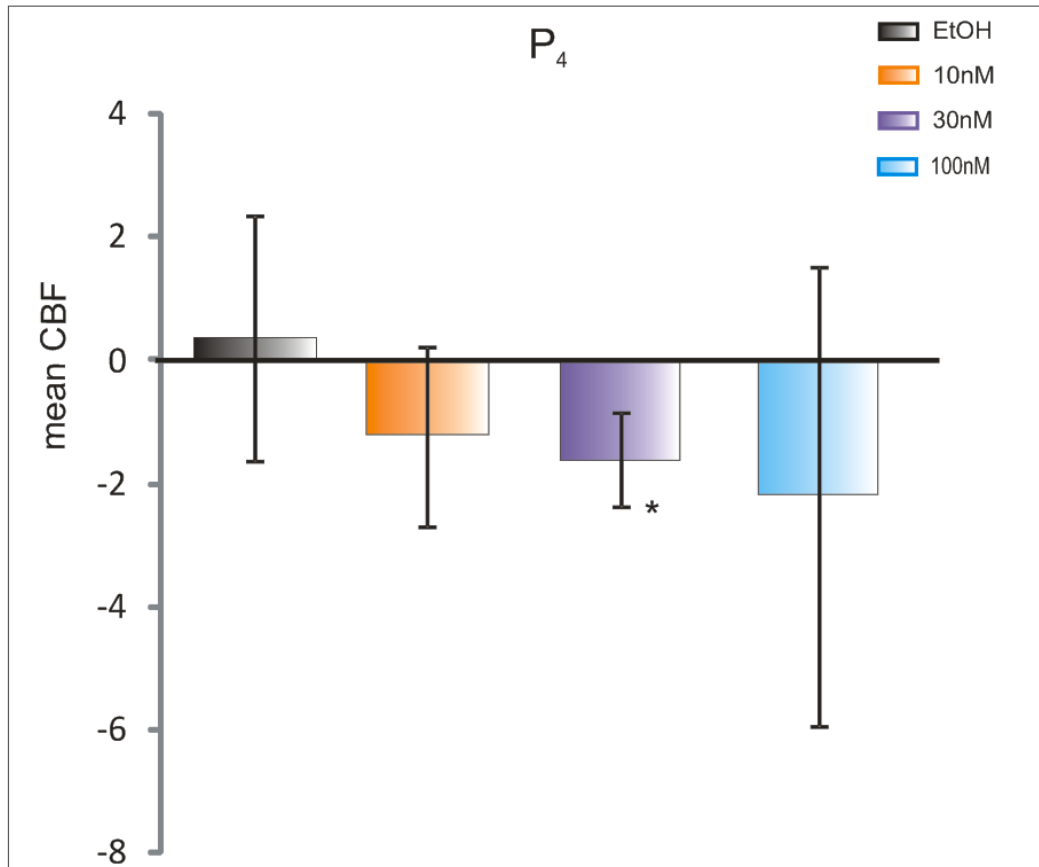


Figure 11: Average change in CBF for cilia in the fallopian tube of mice after treatment with P_4 . The change in CBF is the difference between the average CBF during 30-60 minutes after addition of P_4 and the average CBF for the measurements prior to any treatment. CBFs are presented as mean \pm SEM. Cells exposed to ethanol (EtOH) are used as a control. The significance is shown as * $P < 0.05$.

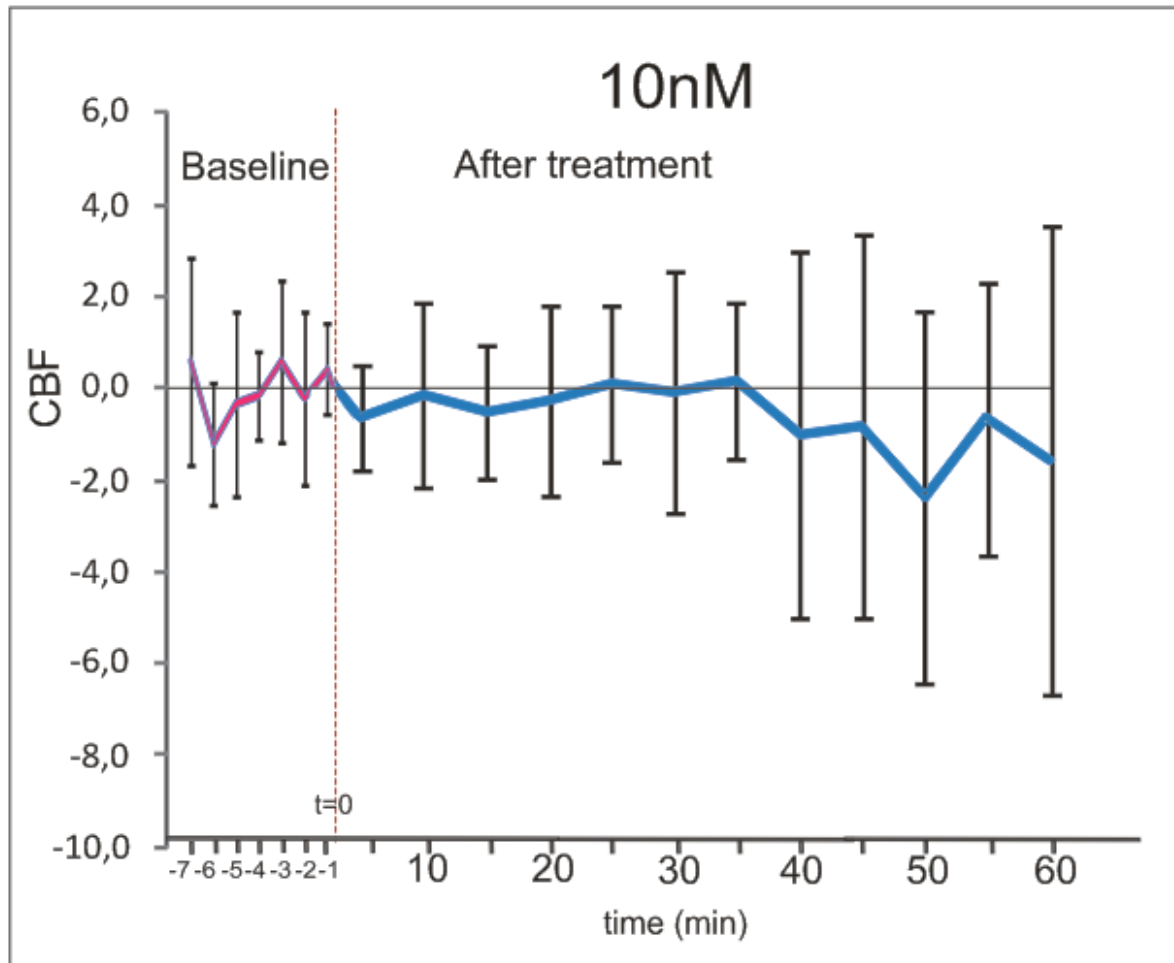


Figure 12: Change in CBF over time for cilia in the fallopian tube of mice after treatment with P_4 of the concentration 10 nM. The baseline represents the 7 CBF measurements prior to treatment. CBF was measured every 5 minute for 60 minutes after addition of P_4 and normalized to the mean CBF prior to treatment. The CBF values presented are normalized to the control cells (treated with ethanol) and expressed in relative numbers to the control groups as mean \pm SEM.

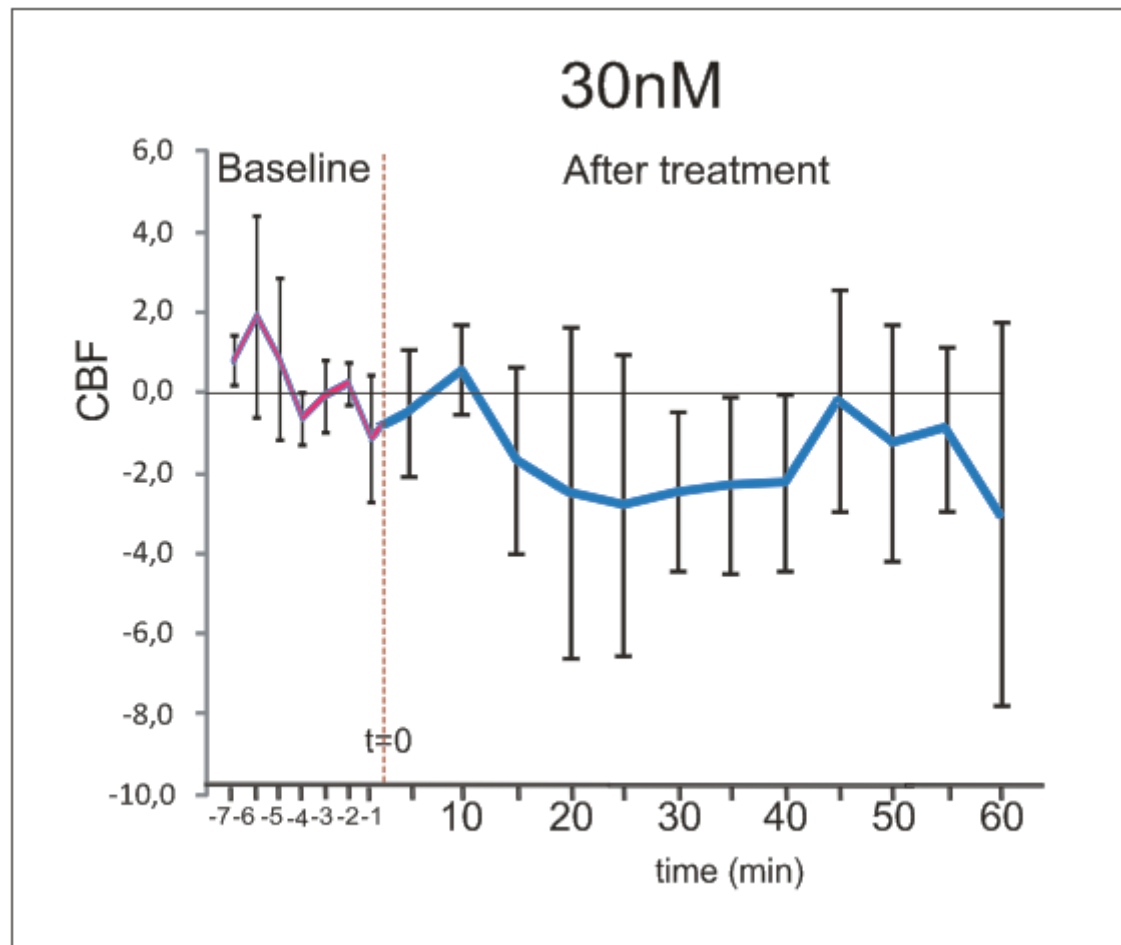


Figure 13: Change in CBF over time for cilia in the fallopian tube of mice after treatment with P_4 of the concentration 30 nM. The baseline represents the 7 CBF measurements prior to treatment. CBF was measured every 5 minute for 60 minutes after addition of P_4 and normalized to the mean CBF prior to treatment. The CBF values presented are normalized to the control cells (treated with ethanol) and expressed in relative numbers to the control groups as mean \pm SEM.

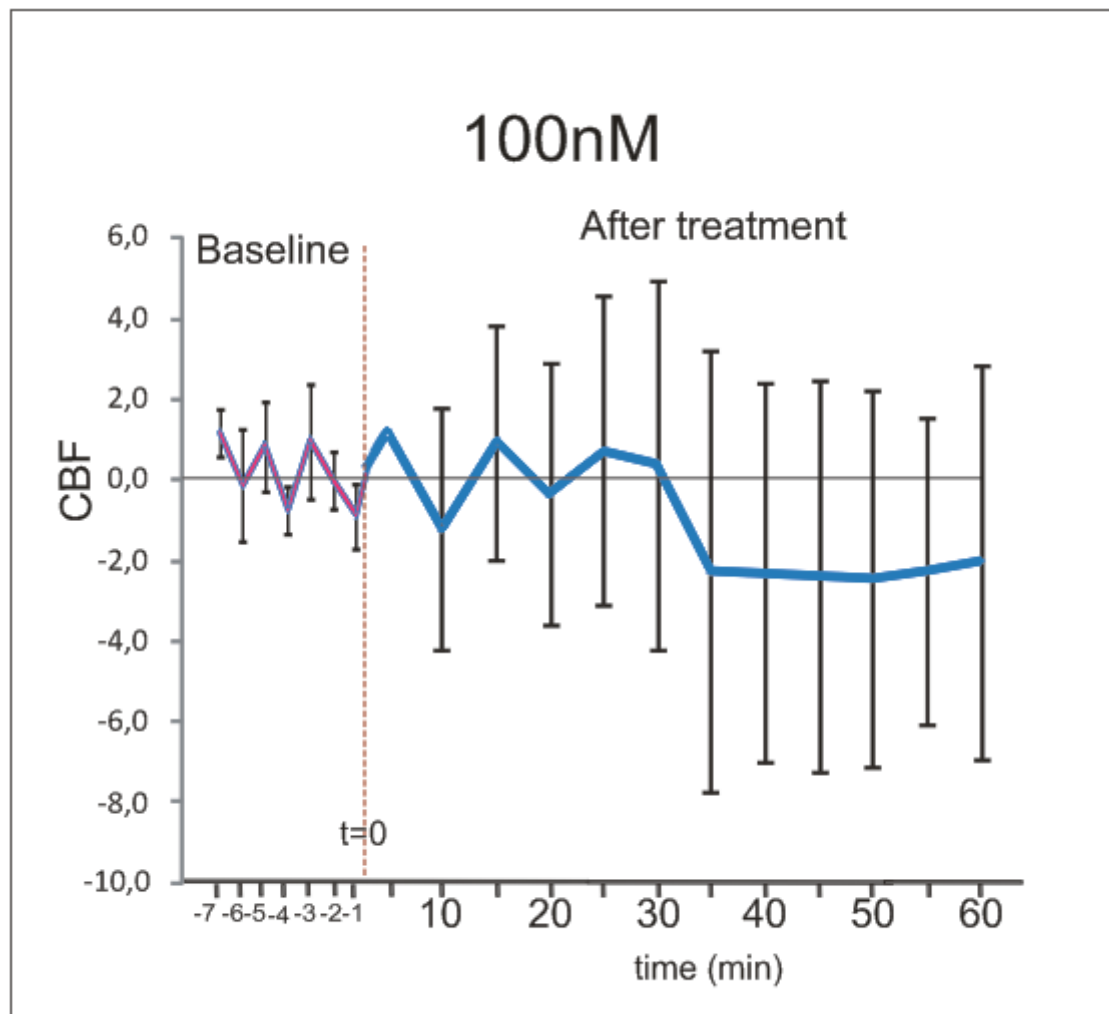


Figure 14: Change in CBF over time for cilia in the fallopian tube of mice after treatment with P_4 of the concentration 100 nM. The baseline represents the 7 CBF measurements prior to treatment. CBF was measured every 5 minute for 60 minutes after addition of P_4 and normalized to the mean CBF prior to treatment. The CBF values presented are normalized to the control cells (treated with ethanol) and expressed in relative numbers to the control groups as mean \pm SEM.

R5020

3 concentrations of the nPR-agonist were tested (10 nM, 100 nM and 500 nM). For each concentration the average change in CBF, when comparing mean CBF prior to treatment with the mean CBF between 30-60 minutes after addition of R5020, was calculated. The average CBF and standard deviation for each concentration and control cells, exposed only to ethanol, is visualized in *Figure 15*. For the concentration 100 nM the reduction in CBF was significant with 3.13 Hz ($p=0.0046$). The low concentration gave an average

increase in CBF (0.23 Hz, $p=0.45$). For R5020 500 nM there was an average decrease in CBF (-1.22 Hz, $p=0.15$).

CBF was measured every 5 minute for 60 minutes after addition of R5020 and normalized to the mean CBF prior to treatment. The CBF values were then normalized to the control cells (treated with ethanol). In *Figure 16* the normalized change in CBF prior to any treatment and after addition of R5020 10 nM is plotted. In *Figure 17* and *Figure 18* the normalized change in CBF prior to and after addition of R5020 100 nM and R5020 500 nM respectively, are plotted.

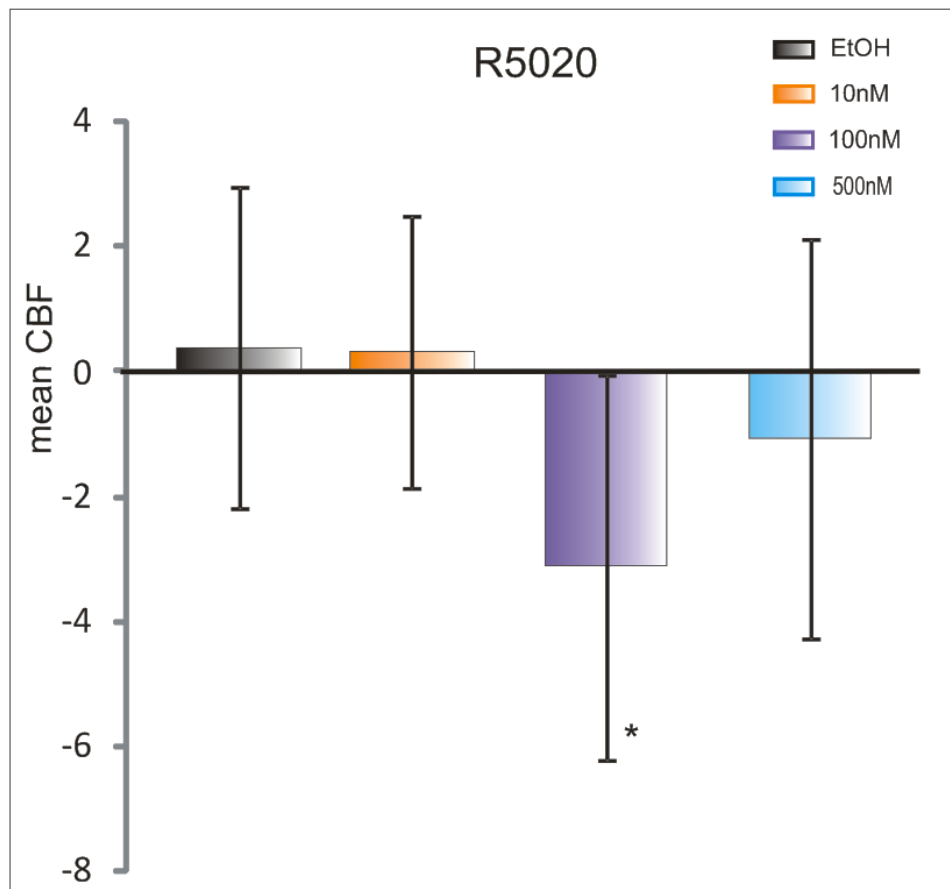


Figure 15: Average change in CBF for cilia in the fallopian tube of mice after treatment with R5020. The change in CBF is the difference between the average CBF during 30-60 minutes after addition of R5020 and the average CBF for the measurements prior to any treatment. CBFs are presented as mean \pm SEM. Cells exposed to ethanol (EtOH) are used as a control. The significance is shown as * $P < 0.05$.

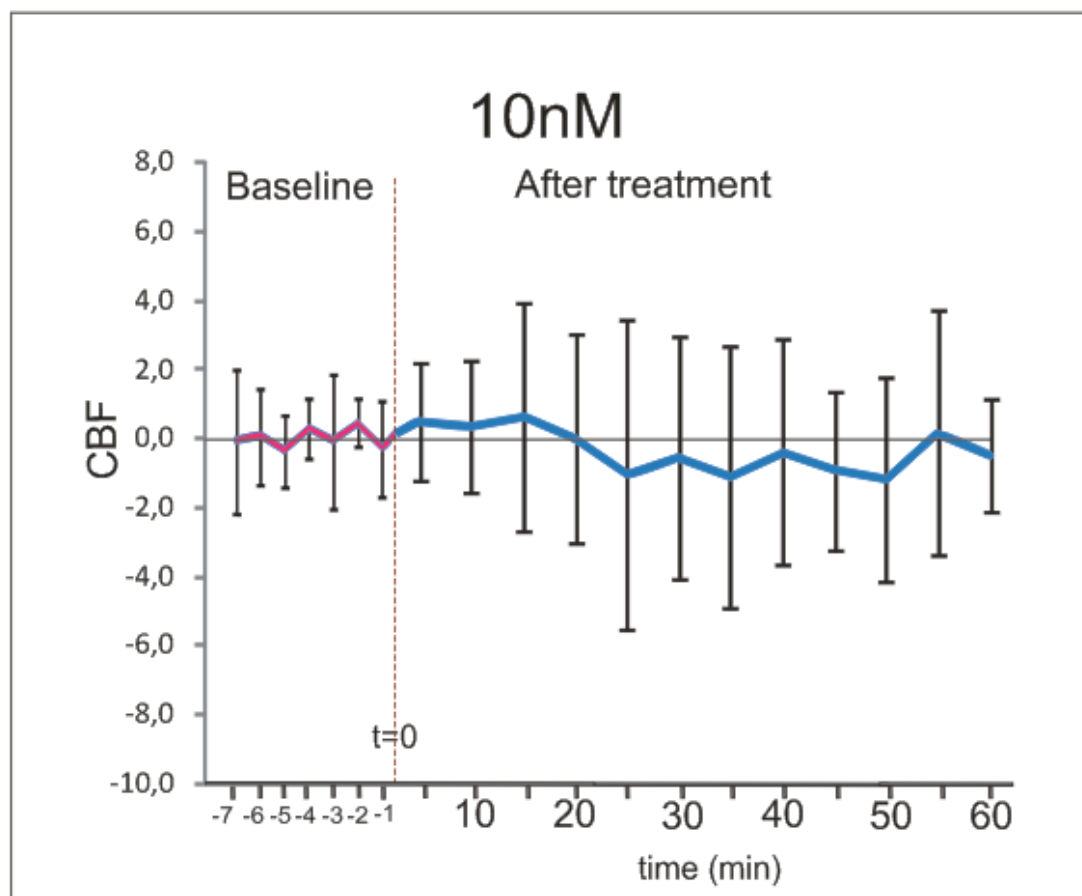


Figure 16: Change in CBF over time for cilia in the fallopian tube of mice after treatment with R5020 of the concentration 10 nM. The baseline represents the 7 CBF measurements prior to treatment. CBF was measured every 5 minute for 60 minutes after addition of R5020 and normalized to the mean CBF prior to treatment. The CBF values presented are normalized to the control cells (treated with ethanol) and expressed in relative numbers to the control groups as mean \pm SEM.

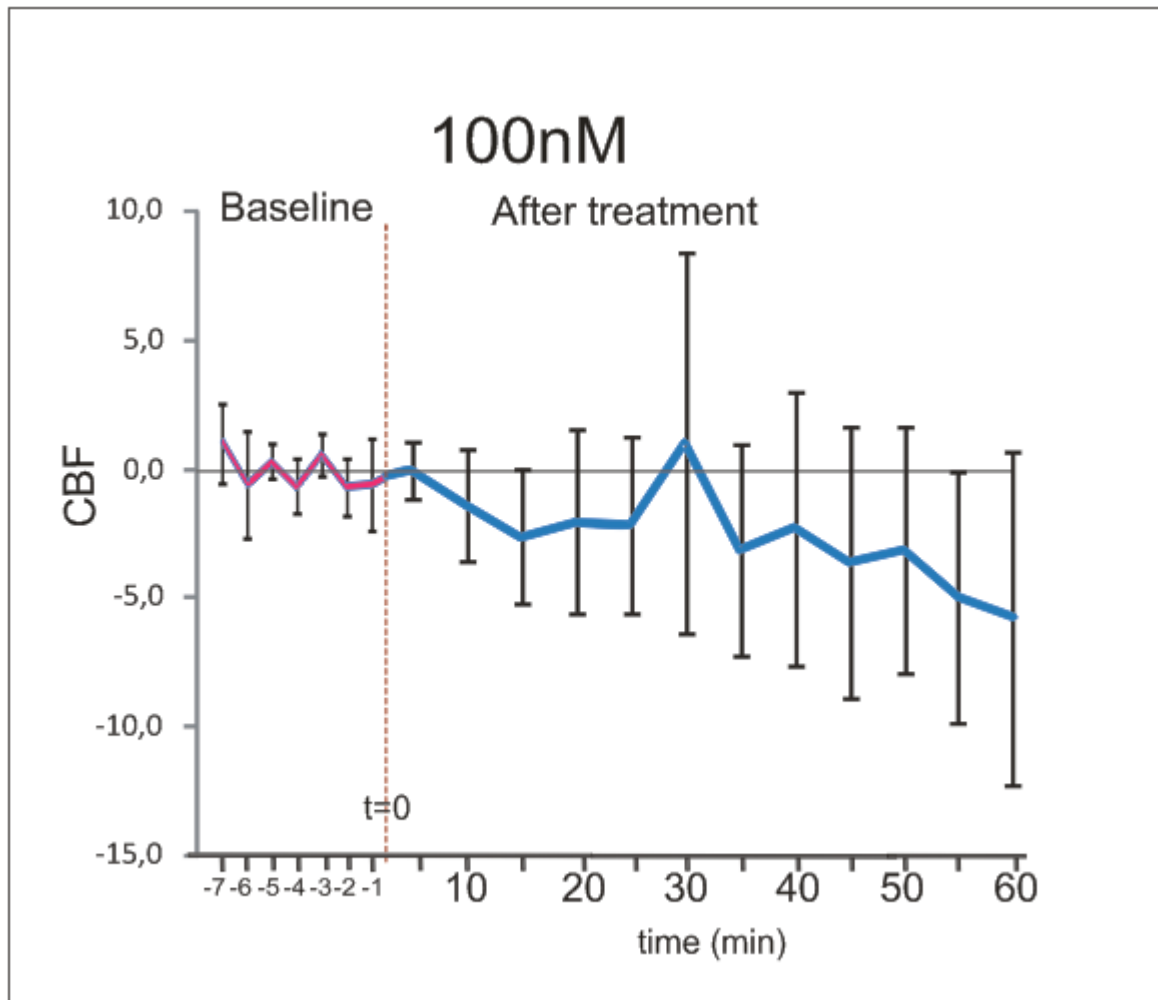


Figure 17: Change in CBF over time for cilia in the fallopian tube of mice after treatment with R5020 of the concentration 100 nM. The baseline represents the 7 CBF measurements prior to treatment. CBF was measured every 5 minute for 60 minutes after addition of R5020 and normalized to the mean CBF prior to treatment. The CBF values presented are normalized to the control cells (treated with ethanol) and expressed in relative numbers to the control groups as mean \pm SEM.

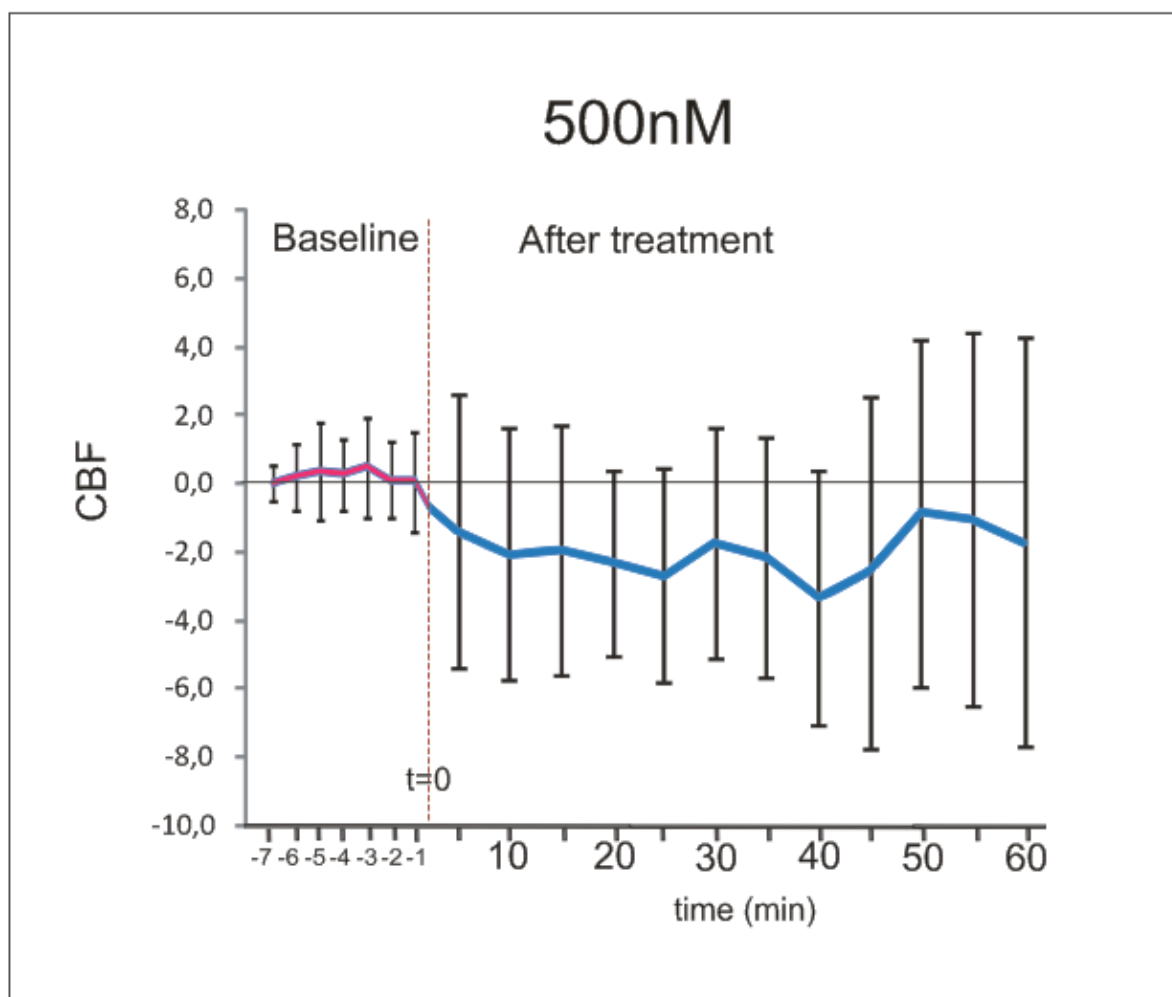


Figure 18: Change in CBF over time for cilia in the fallopian tube of mice after treatment with R5020 of the concentration 500 nM. The baseline represents the 7 CBF measurements prior to treatment. CBF was measured every 5 minute for 60 minutes after addition of R5020 and normalized to the mean CBF prior to treatment. The CBF values presented are normalized to the control cells (treated with ethanol) and expressed in relative numbers to the control groups as mean \pm SEM.

Substance X

Four different concentrations of substance X were tested (10 nM, 100 nM, 500 nM and 2.5 μ M). For each concentration the average change in CBF, when comparing mean CBF prior to treatment with the mean CBF between 30-60 minutes after addition of substance X, was calculated. The average CBF and standard deviation for each concentration and control cells, exposed only to ethanol, is visualized in *Figure 19*. None of the concentrations showed significance in changed CBF. There was an average increase in CBF for every concentration compared with controls. The low concentration 10 nM

gave an average increase of 2.25 Hz ($p=0.13$), 100 nM gave an average increase of 1.83 Hz ($p=0.49$), 500 nM gave an average increase of 0.23 Hz ($p=0.37$) and finally 2.5 μ M gave an average increase of 3.42 ($p=0.16$).

CBF was measured every 5 minute for 60 minutes after addition of substance X and normalized to the mean CBF prior to treatment. The CBF values were then normalized to the control cells (treated with ethanol). In *Figure 20, 21, 22 and 23* the normalized change in CBF prior to treatment and after addition of substance X with concentrations 10 nM, 100 nM, 500 nM and 2.5 μ M respectively, are plotted.

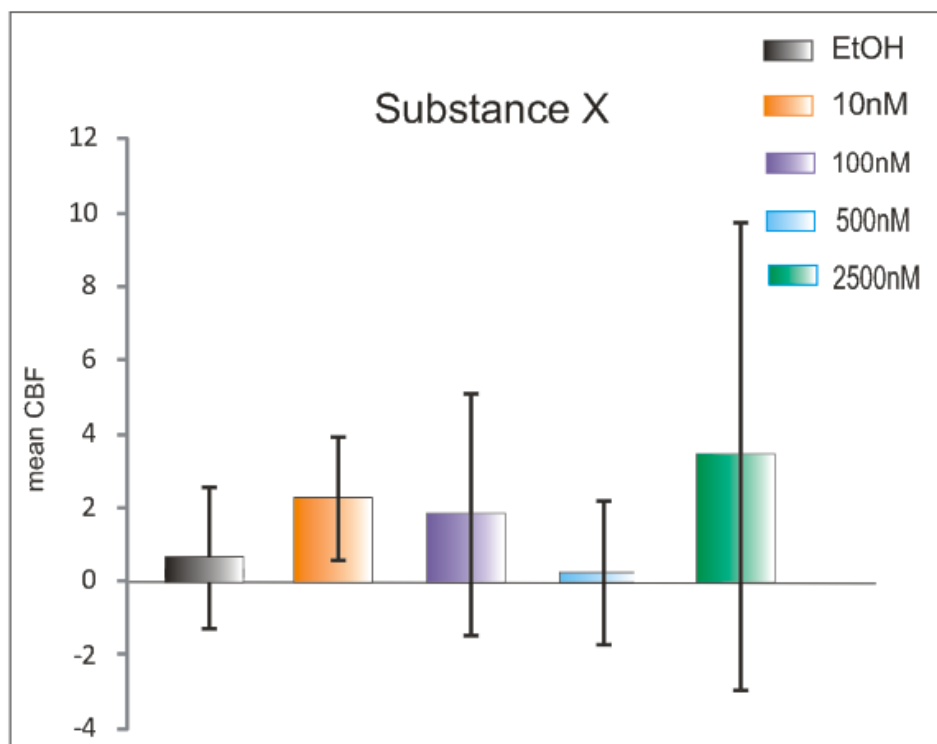


Figure 19: Average change in CBF for cilia in the fallopian tube of mice after treatment with substance X. The change in CBF is the difference between the average CBF during 30-60 minutes after addition of X and the average CBF for the measurements prior to any treatment. CBFs are presented as mean \pm SEM. Cells exposed to ethanol (EtOH) are used as a control.

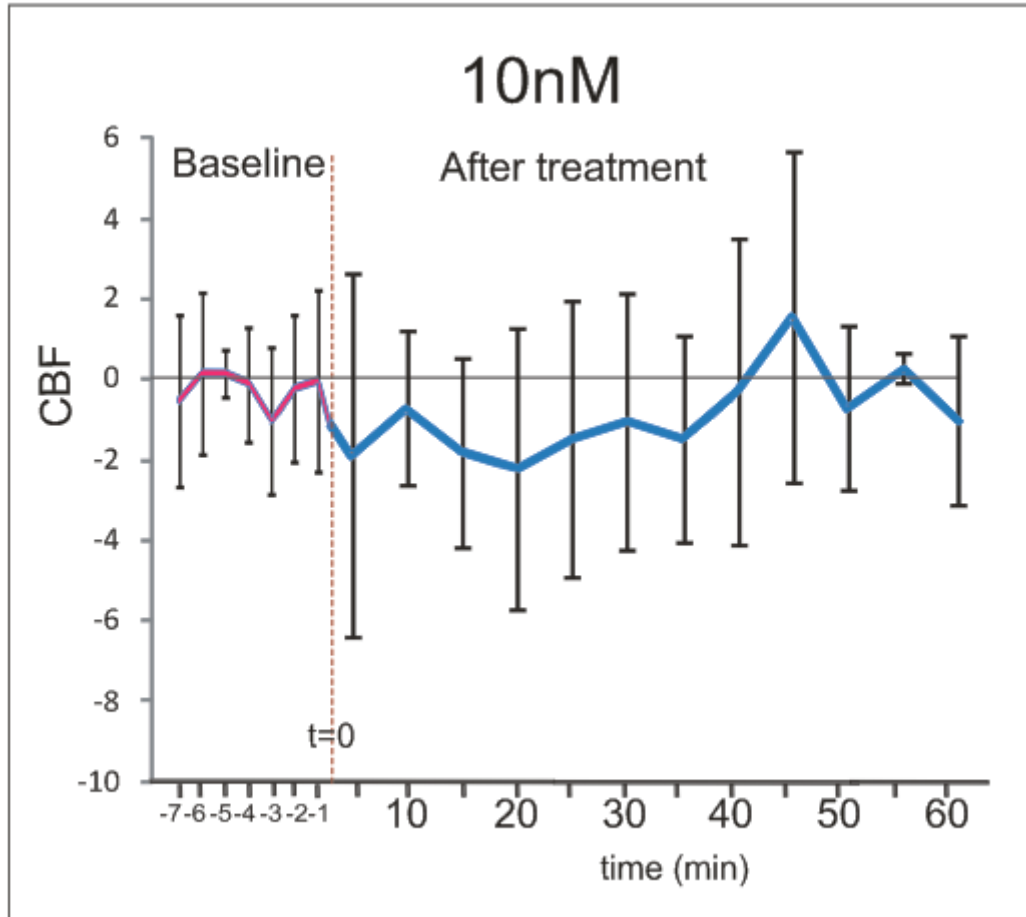


Figure 20: Change in CBF over time for cilia in the fallopian tube of mice after treatment with substance X of the concentration 10 nM. The baseline represents the 7 CBF measurements prior to treatment. CBF was measured every 5 minute for 60 minutes after addition of substance X and normalized to the mean CBF prior to treatment. The CBF values presented are normalized to the control cells (treated with ethanol) and expressed in relative numbers to the control groups as mean \pm SEM.

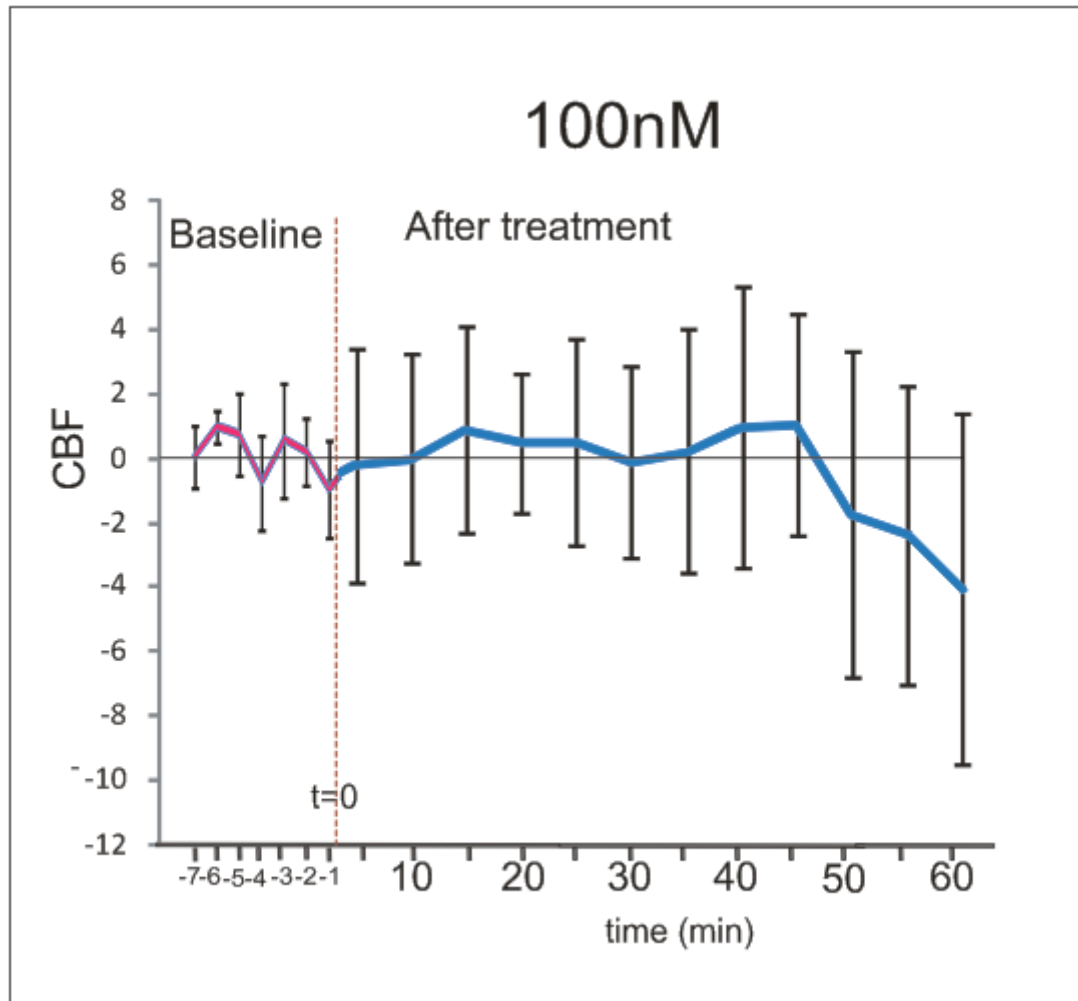


Figure 21: Change in CBF over time for cilia in the fallopian tube of mice after treatment with substance X of the concentration 100 nM. The baseline represents the 7 CBF measurements prior to treatment. CBF was measured every 5 minute for 60 minutes after addition of substance X and normalized to the mean CBF prior to treatment. The CBF values presented are normalized to the control cells (treated with ethanol) and expressed in relative numbers to the control groups as mean \pm SEM.

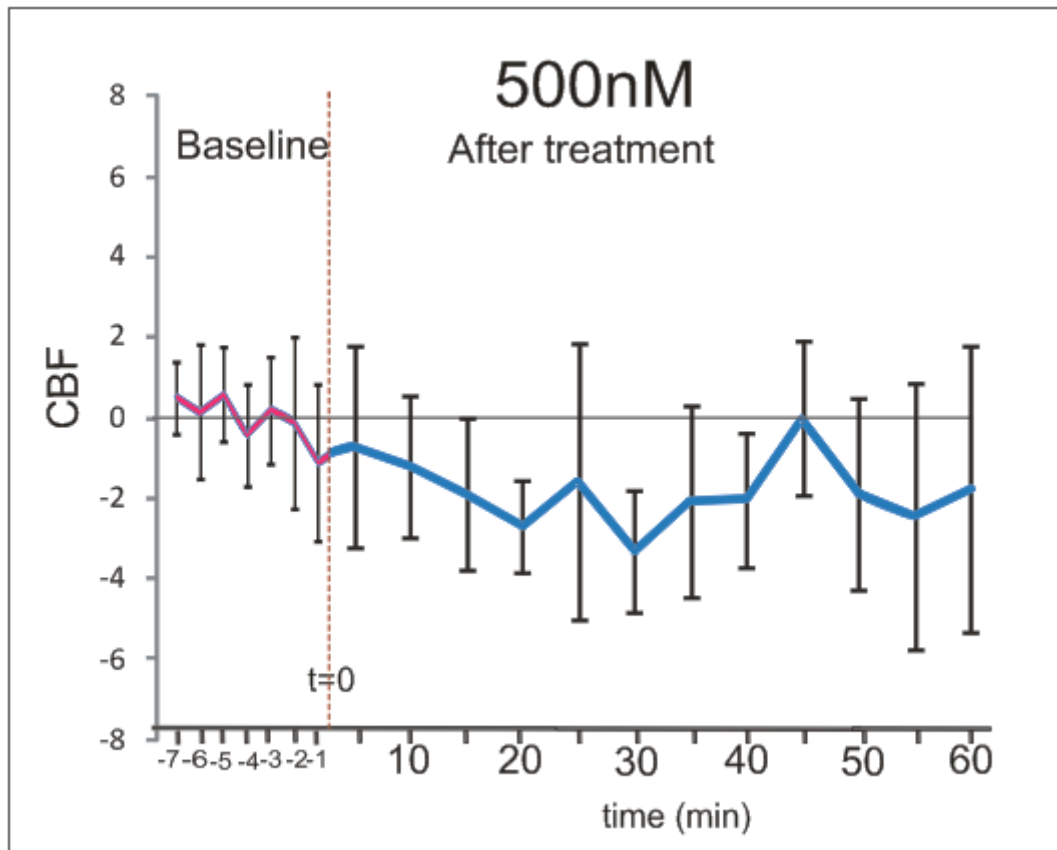


Figure 22: Change in CBF over time for cilia in the fallopian tube of mice after treatment with substance X of the concentration 500 nM. The baseline represents the 7 CBF measurements prior to treatment. CBF was measured every 5 minute for 60 minutes after addition of substance X and normalized to the mean CBF prior to treatment. The CBF values presented are normalized to the control cells (treated with ethanol) and expressed in relative numbers to the control groups as mean \pm SEM.

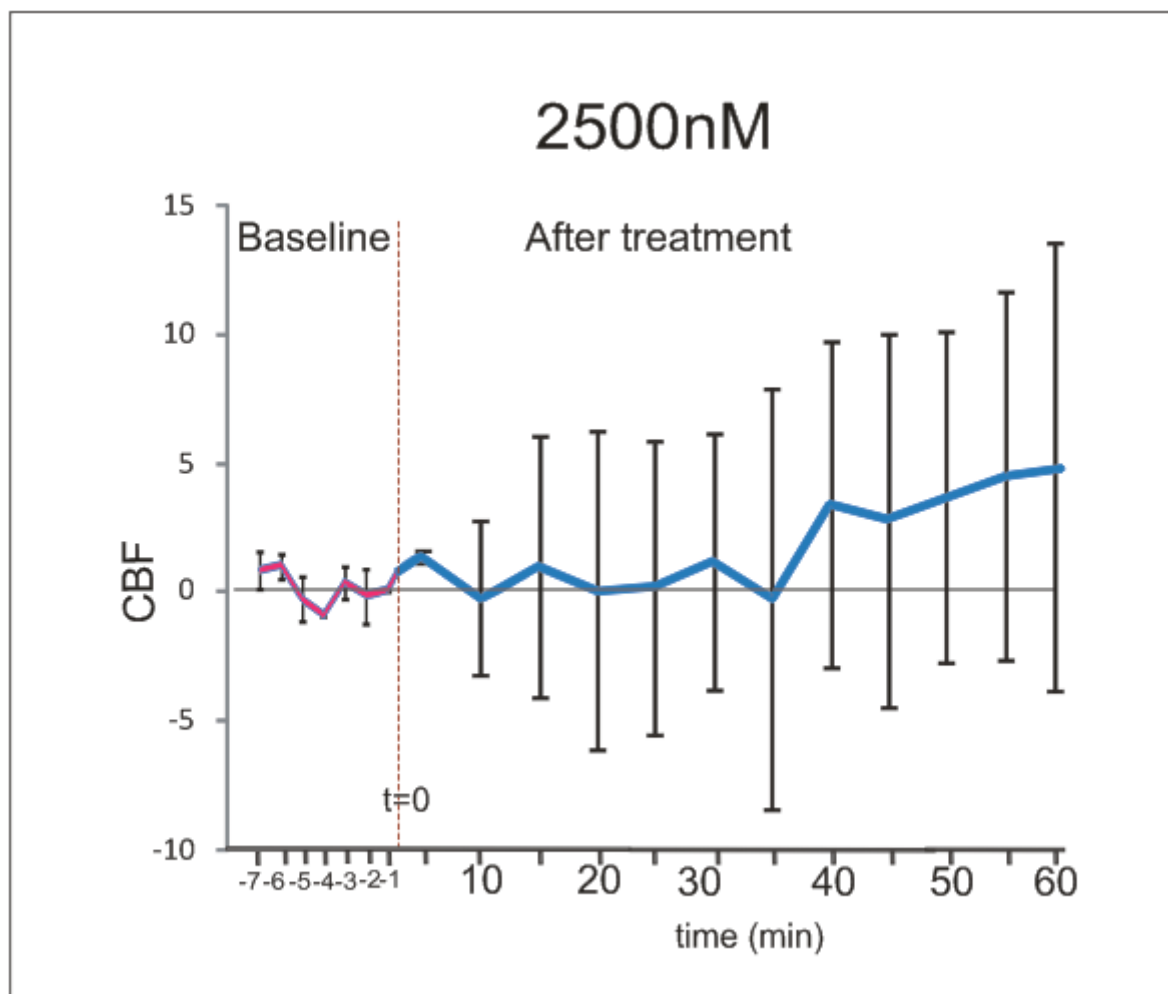


Figure 23: Change in CBF over time for cilia in the fallopian tube of mice after treatment with substance X of the concentration 2500 nM. The baseline represents the 7 CBF measurements prior to treatment. CBF was measured every 5 minutes for 60 minutes after addition of substance X and normalized to the mean CBF prior to treatment. The CBF values presented are normalized to the control cells (treated with ethanol) and expressed in relative numbers to the control groups as mean \pm SEM.

RU486 mifepristone

Only one concentration of mifepristone was tested (1 μ M). CBF was measured for 90 minutes after addition of mifepristone. The average change in CBF, when comparing mean CBF after treatment with the mean CBF between 30-60 minutes after addition of RU486, was calculated. The average CBF and standard deviation for this concentration and control cells, exposed only to ethanol, is visualized in *Figure 24*. The average change in CBF,

+0.7 Hz was not significant ($p=0.40$) for this concentration.

CBF was measured every 5 minutes for 90 minutes after addition of RU486 and normalized to the mean CBF prior to treatment. The CBF values were then normalized to the control cells (treated with ethanol). In *Figure 25* the normalized change in CBF prior to any treatment and after addition of RU486 1 μ M is plotted. No trend in changed CBF can be seen.

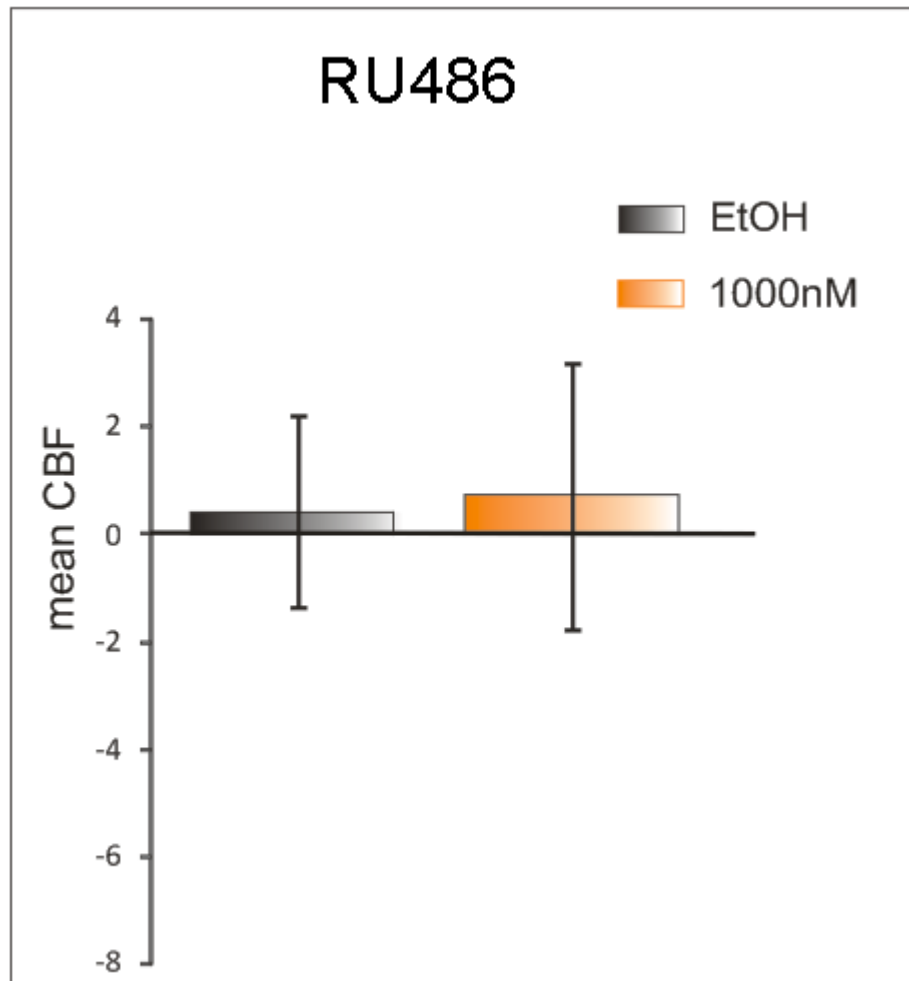


Figure 24: Average change in CBF for cilia in the fallopian tube of mice after treatment with RU486. The change in CBF is the difference between the average CBF during 30-60 minutes after addition of RU486 and the average CBF for the measurements prior to any treatment. CBFs are presented as mean \pm SEM. Cells exposed to ethanol (EtOH) are used as a control.

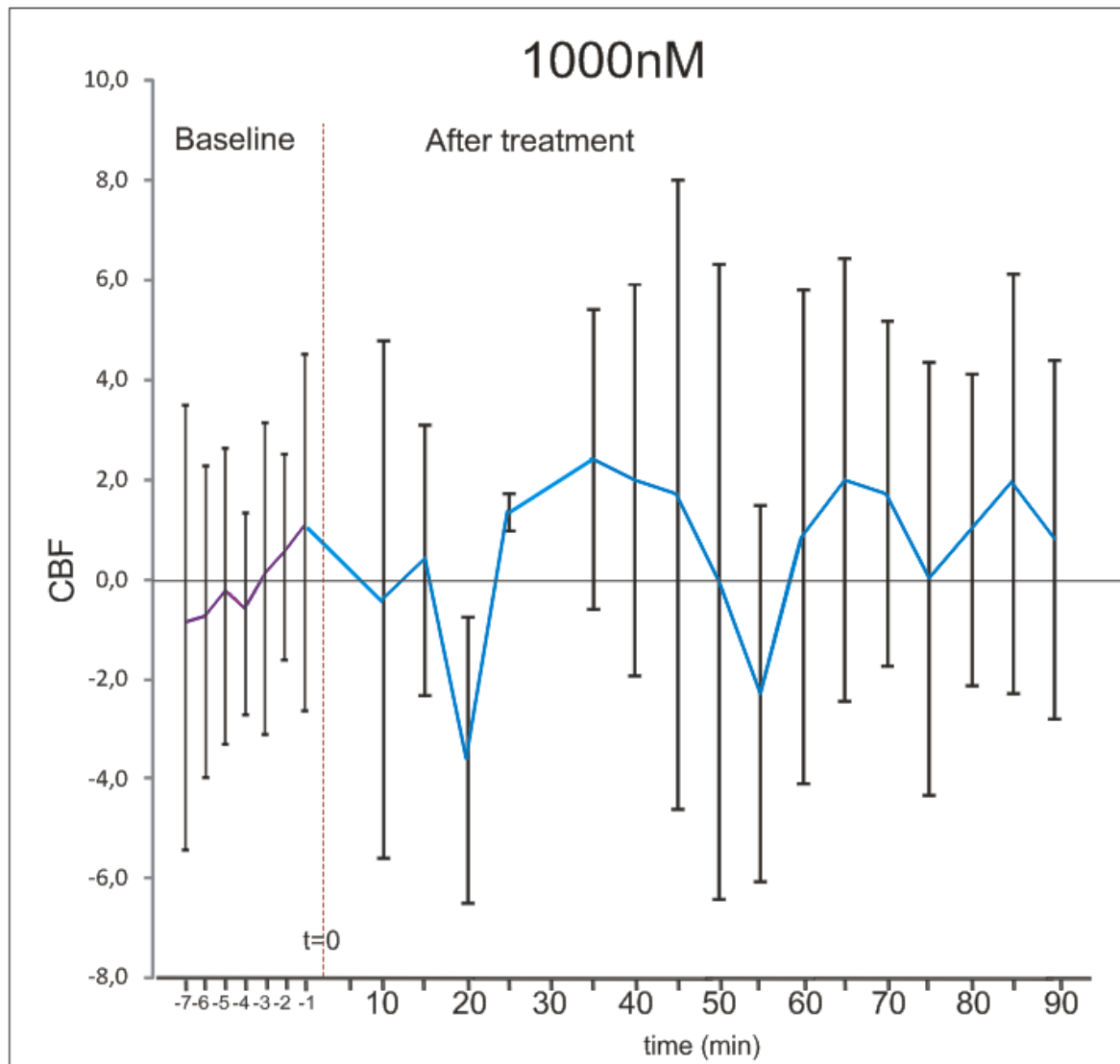


Figure 25: Change in CBF over time for cilia in the fallopian tube of mice after treatment with RU486 of the concentration 1000 nM. The baseline represents the 7 CBF measurements prior to treatment. CBF was measured every 5 minute for 90 minutes after addition of RU486 and normalized to the mean CBF prior to treatment. The CBF values presented are normalized to the control cells (treated with ethanol) and expressed in relative numbers to the control groups as mean \pm SEM.

DISCUSSION

Progesterone with the concentration 30 nM gave a significant decrease in CBF. A reduction in CBF after treating the cells with progesterone have been shown in previous studies performed on humans [1], bovine [46] and mice [5]. Progesterone with the concentrations 10 nM and 100 nM also gave a reduction in CBF even though not statistically significant. Progesterone acts as an agonist for both the nPR and mPR and it cannot be concluded through

which receptor progesterone exerts its effect.

A significant decrease in CBF was seen for R5020 with the concentration 100 nM. The low concentration, 10 nM, gave an increase in CBF and the high concentration, 500 nM, gave a decrease in CBF. These results were not significant. R5020 works as an agonist for the nPR and binds only with low affinity to the mPR. The significant result for a decrease in CBF suggests that the nuclear progesterone

receptor is involved in the fast response in CBF seen by progesterone

Substance X is used as an agonist for the progesterone membrane receptor. The 4 concentrations tested showed no significant effect but there was an average increase in CBF for every concentration. This might indicate that the membrane receptor is not involved in the regulation of CBF in a fast response.

Mifepristone with the concentration 1 μM showed an average increase of 1.7 Hz on CBF. The control cells showed an average increase of 0.53 Hz. This difference was not statistically different. Mifepristone acts as a partial agonist for the nPR-B and an antagonist for the nPR-A and should give similar effects as progesterone.

The baseline CBF before treatment showed a large variation between different samples, ranging from 13 to 44 Hz with an average of 29.2 Hz. This agrees rather well with the mean CBF, 22.5 Hz, obtained from the study performed by Bylander *et al.* [5]. Shi *et al.* [14] calculated the average CBF to 10.9 Hz during the diestrus stage and 8.5 Hz during the estrus stage in their study. The variation in baseline CBF between different studies can have several explanations. Mahmood *et al.* [1] have seen a significant difference in CBF between two different segments in human fallopian tubes. In the ampullary segment the average CBF was 5.4 Hz and in the fimbrial segment the average CBF was 4.8 Hz [1]. In a study performed by Wessel *et al.* [39] it was conducted that the sample temperature was an important factor giving an increase in CBF by 0.9 Hz/ $^{\circ}\text{C}$. In our experiments the tissue samples were kept at a constant temperature of 36.5 $^{\circ}\text{C}$ to represent the body temperature.

Preparation of the tissue and the condition during the experiments can also give explanation for the variation in CBF between this study and the study performed by Shi *et al.* [14]. A possible explanation for the large difference is that the mice in this study are immature while

Shi *et al.* uses mice going through the estrous cycle. Shi *et al.* also uses a different detection method and the samples are collected and measured within 15 minutes and not affected by any cultivation medium [14].

Nutu *et al.* [32] used specific antibodies to detect the presence of mPRs in the reproductive tract in mouse. mPR γ and mPR β were both found to be expressed within the cilia in the fallopian tube. mPR γ was found at the base of the cilia while mPR β was found on the motile cilia. It was also shown that the gene expression of mPR β and γ was down-regulated by progesterone in gonadotropin-primed mice. The presence of mPRs could provide a mechanism to the rapid effect seen by progesterone on tissues lacking the nuclear receptor [32].

Teilmann *et al.* [21] detected nuclear progesterone receptors on the base of the cilia in the fallopian tube of mouse. They suggest that these receptors are involved in a fast non-genomic response on ciliary activity by progesterone. Present at the cilia base are also effector molecules that are believed to mediate the effect of progesterone [21].

In a study performed by Wessel *et al.* [46] it was investigated how pretreatment with mifepristone followed by exposure to progesterone affected CBF of bovine oviducts. The cells were pretreated with a 20 μM concentration of mifepristone for 2 hours. They were then exposed to progesterone with a concentration of 20 μM . Another series of experiments was also carried out where the cells were exposed to only progesterone with the concentration 20 μM . The CBF was measured every 15 minutes for 2 hours. The two sets of experiments showed the same results with a reduction of 11 % in CBF after 2 hours and a reduction in CBF could be seen after only 15 minutes. The results indicate that the rapid change in CBF caused by progesterone is not regulated by the nuclear progesterone

receptor. Additional measurements that were carried out 24 hours after treatment showed maintained CBF reduction in the cells treated by progesterone alone. In the cells pretreated by mifepristone there was no CBF inhibition compared to control [46]. Mifepristone has been shown to have a higher affinity for the nuclear progesterone receptor compared to progesterone [38]. One possibility is that mifepristone has outcompeted progesterone for the receptor after 24 hours and thus the loss of CBF inhibition.

Mahmood *et al.* [1] performed a similar study as Wessel *et al.* [46] with fallopian tubes from women. In one group the tissue sample was exposed to only progesterone with concentrations ranging from 0.1 μM to 10 μM and the other exposed for progesterone and mifepristone together at a concentration of 10 μM . The samples were incubated for 24 hours before measurements of CBF. In cells exposed to only progesterone CBF was reduced. The inhibition was dependent on concentration and the higher concentration resulted in a greater reduction. In the cells exposed to both mifepristone and progesterone the reduction of CBF was reversed [1]. This is consistent with the theory that progesterone exerts its effect on CBF with involvement of nPR.

The cilia have proven to be rather sensitive to changes in the environment [4] and to minimize errors all experiments have been performed in the same manner in the largest extent possible. To avoid losing the sample when adding hormone steroids to the dish the sample is attached to the bottom by a needle. The baseline CBF is measured after attachment of the needle to provide the same conditions for each measuring. The tissue samples are very small and can move under the needle giving inaccurate measuring that cannot be used. An improvement of the method could be developed to ease for future measuring of the CBF.

CONCLUSION

In summary, the method described can be used to investigate the CBF in the fallopian tube of mice.

Progesterone with a concentration of 30 nM has been shown to significantly decrease CBF in a rapid response. This corresponds well with previous studies performed on mice with other concentrations [5]. Two other concentrations (10 nM and 100 nM) of progesterone were tested and gave a decrease in CBF. This reduction was however not statistically significant.

Four concentrations of the mPR agonist, substance X, were tested and gave no significant change in CBF.

R5020 with the concentration 100 nM gave a significant decrease in CBF while the other concentrations (10 nM and 500 nM) gave no significant change in CBF. R5020 works as an agonist for the nPR and thus the result obtained in this study indicate that CBF is regulated by nPR and not mPR.

FUTURE WORK

The method used, to investigate the CBF of fallopian tube in mice, in this report can be further improved. Today the system is open leading to an evaporation of liquid. This was evident in the case where the measurements lasted for about 1,5 hours for mifepristone. The loss of liquid led to reduced ciliary activity. For these measurements 2 mL of liquid was used. It was considered to put a lid over the petri dish but then it would be hard to attach the tissue sample with the needle. A continuous system providing a constant liquid level in the dish will give more reliable measurements

RU486 is an antagonist to the nuclear progesterone receptor and has, based on the experiments carried out in this project,

no effect on CBF. By pretreating the cells with RU486 (1 μ M) for 30 min the nuclear progesterone receptor should be blocked. The treated cells should then be incubated with progesterone of a concentration (30 nM) that has proven to have significant effect on CBF. By measuring CBF it can be concluded if the nPR is involved in the fast response in changed average CBF seen with progesterone.

Future studies should involve knock-out mice missing the gene coding for the nuclear progesterone receptor. The fallopian tube of these mice will be treated with progesterone of a concentration that has proven to have significant effect on CBF. This setup has good potential to provide further evidence of which progesterone receptor (or receptors) that is involved in the fast response.

ACKNOWLEDGEMENT

There are many people that have contributed to the final result of this

project. I would like to give a special thanks to:

Anna Bylander, my supervisor during the experimental part of the thesis. Thank you for great support and guidance during the project.

Joakim Larsson, my supervisor at sahlgrenska academy for all the encouragement and input during the project.

Mattias Goksör at the department of physics for all the help with the microscope during the measurements and how to interpret the results.

Christer Larsson, my examiner at Chalmers.

I would also like to thank the personnel at the institute of Neuroscience and Physiology and department of physics that has been involved in my project.

Finally I would like to thank my family and friends that has supported and encouraged me during this work.

REFERENCES

- [1] Mahmood, T., Saridogan, E., Smutna, S., Habib, A.M., Djahanbakeh, O. (1998) "The effect of ovarian steroids on epithelial ciliary beat frequency in the human Fallopian tube" *Human Reproduction* Vol. 13 (11), pp. 2991-2994.
- [2] Paltieli, Y., Eibschitz, I., Ziskind, G., Ohel, G., Silbermann, M and Weichselbaum, A. (1999) "High Progesterone Levels and Ciliary Dysfunction- A Possible Cause of Ectopic Pregnancy" *Journ. of Ass. Repr. and Genetics*, Vol. 17 (2), pp. 104-106.
- [3] Lyons, R.A., Saridogan, E., Djahanbakeh, O. (2006) "The reproductive significance of human Fallopian tube cilia" *Human Reproduction Update* Vol. 12 (4), pp. 363-372.
- [4] Nutu, Magdalena (2009) "The role of progesterone in the regulation of ciliary activity in the fallopian tube" University of Gothenburg. Accessible at: http://gupea.ub.gu.se/bitstream/2077/21415/4/gupea_2077_21415_4.pdf [2011-01-21]
- [5] Bylander, A., Nutu, M., Wellander, R., Goksör, M., Billig, H., Larsson, J. (2010) "Rapid effects of progesterone on ciliary beat frequency in the mouse fallopian tube". Accessible at: <http://www.rbej.com/content/8/1/48> [2011-01-21]
- [6] El-Mowafi, D.M., Diamond, M.P. (2008) "Fallopian tube" Accessible at: http://www.gfmer.ch/International_activities_En/El_Mowafi/Fallopian_tube.htm [2011-02-25]
- [7] Slomianka, Lutz (2009) "Blue Histology – Female Reproductive System" <http://www.lab.anhb.uwa.edu.au/mb140/CorePages/FemaleRepro/femalerepro.htm> [2011-03-21]
- [8] Blaustein, A and Kurman, R.J. (2002) "Balustein's pathology of the female genital tract" New York: Springer-Verlag.
- [9] Satir, P., Christensen, S.T. (2007) "Overview of Structure and Function of Mammalian Cilia" *Annu. Rev. Physiol.* Vol 69, pp. 377-400.
- [10] Karteris, E., Zervou, S., Pang, Y., Dong, J., Hillhouse, E.W., Randewa, H.S and Thomas, P. (2006) "Progesterone signalling in human myometrium through two novel membrane G protein-coupled receptors: potential role in functional progesterone withdrawal at term" *Mol. Endocrinol.*, Vol. 20, pp. 1519-1534.
- [11] Thai, C.H., Gambling, T.M., Carson, J.L. (2002) "Freeze fracture study of airway epithelium from patients with primary ciliary dyskinesia" *Thorax*, Vol. 57, pp. 363-365
- [12] Croxatto, H. (2002) "Physiology of gamete and embryo transport through the Fallopian tube" *Reproductive Biomedicine*, Vol. 4 (2), pp. 160-169.

- [13] Lyons, R.A., Saridogan, E., Djahanbakhch, O. (2006) "The effect of ovarian follicular fluid and peritoneal fluid on Fallopian tube ciliary beat frequency" *Human Reproduction* Vol. 21 (1), pp. 52-56.
- [14] Shi, D., Komatsu, K., Uemura, T. and Fujimori, T. (2011) "Analysis of ciliary beat frequency and ovum transport ability in the mouse oviduct" *Genes to cells*, Vol. 16, pp. 282-290.
- [15] Paltieli, Y., Eibschitz, I., Ziskind, G., Ohel, G., Silbermann, M., Weichselbaum, A. (1999) "Gynecology" *Journal of Assisted Reproduction and Genetics* Vol. 17 (2), pp. 103-106.
- [16] Rosenblatt, P.L. (2007) "Menstrual Cycle" Accessible at: <http://www.merckmanuals.com/home/au/sec22/ch241/ch241e.html> (2011-04-11)
- [17] Womak, L. (2011) "Birth control and the female athlete" Accessible at: http://www.sportsmd.com/SportsMD_Articles/id/309.aspx (2011-08-02)
- [18] "Healthy Woman Series: Menstrual Cycles" Accessible at: <http://www.humanbodydetectives.com/blog/2011/05/healthy-woman-series-menstrual-cycles/> (2011-08-05)
- [19] Deb, K., Reese, J., Paria, C.B. (2005) "Methodologies to Study Implantation in Mice" Accessible at: http://vuneo.org/Reese02_Deb_7_34.pdf (2011-04-19)
- [20] Hill, Mark. (2008) "Mouse Development- Estrous Cycle" UNSW Embryology ISBN: 978 0 7334 2609 4. Accessible at: <http://embryology.med.unsw.edu.au/OtherEmb/mouse2.htm> (2011-04-19)
- [21] Teilman, S.C., Clement, C.A., Thorup, J., Byskov, A.G., Christensen, S.T. (2006) "Expression and localization of the progesterone receptor in mouse and human reproductive organs" *Journal of Endocrinology*, Vol. 191, pp. 525-535.
- [22] Graham, J.D., Clarke, C.L (1997) "Physiological Action of Progesterone in Target Tissues" *Endoc. Rev.*, Vol. 18, pp. 502-519.
- [23] "Progesteron" Accessible at: http://www.fass.se/LIF/produktfakta/substance_products.jsp?substanceId=IDE4POBUU91NDVERT1 (2011-05-12)
- [24] Nutu, M., Weidegård, B., Thomas, P., Bergh, C., Thurin-Kjellberg, A., Pang, Y., Billig, H., Larsson, J. (2007) "Membrane Progesterone Receptor Gamma: Tissue Distribution and Expression in Ciliated Cells in the Fallopian Tube" *Molecular Reproduction and development*, Vol. 74, pp. 843-850.
- [25] Bain, D.L., Heneghan, A.F., Connaghan-Jones, K.D, Miura, M.T. (2007) "Nuclear Receptor Structure: Implications for Function" *Annu. Rev. Physiol.*, Vol. 69, pp. 201-220.

- [26] Shao, R., Weijdegård, B., Ljungström, K., Friberg, A., Zhu, C., Wang, X., Zhu, Y., Fernandez-Rodriguez, J., Egecioglu, E., Rung, E., Billig, H. (2006) "Nuclear progesterone receptor A and B isoforms in mouse fallopian tube and uterus: implications for expression, regulation, and cellular function" *Am J Physiol Endocrinol Metab*, Vol. 291, pp. 59-72.
- [27] Kraus, W.L and Katzenellenbogen, B.S. (1993) "Regulation of progesterone receptor gene expression and growth in the rat uterus: modulation of estrogen actions by progesterone and sex steroid hormone antagonists" *Endocrinology*, Vol. 132(6), pp. 2371-2379.
- [28] Boonyaratanakornit, V., Scott, M.P., Ribon, V., Sherman, L., Andersson, S.M., Maller, J.L., Miller, W.T. (2001) "Progesterone Receptor Contains a Proline-Rich Motif that Directly Interacts with SH3 Domains and Activates c-Src Family Tyrosine Kinases" *Molecular Cell*, Vol. 8, pp. 269-280.
- [29] Boonyaratanakornit, V., McGowan, E., Sherman, L., Edwards, M.A. (2007) "The Role of Extranuclear Signaling Actions of Progesterone Receptor in Mediating Progesterone Regulation of Gene Expression and the Cell Cycle" *Molecular Endocrinology*, Vol. 21(2), pp. 359-375.
- [30] Zhu, Y., Rice, C.D., Pang, Y., Pace, M., Thomas, P. (2003) "Cloning, expression, and characterization of a membrane progesterin receptor and evidence it is an intermediary in meiotic maturation of fish oocytes" *Proc Natl Acad Sci U S A*, Vol. 100(5), pp. 2231-2236.
- [31] Zhu, Y., Bond, J., Thomas, P. (2003) "Identification, classification, and partial characterization of genes in humans and other vertebrates homologous to a fish membrane progesterin receptor" *Proc Natl Acad Sci U S A*, Vol. 100(5), pp. 2237-2242.
- [32] Nutu, M., Weijdegård, B., Thomas, P., Thurin-Kjellberg, A., Billig, H., Larsson, J. (2009) "Distribution and hormonal regulation of membrane progesterone receptors β and γ in ciliated epithelial cells of mouse and human fallopian tubes" *Reproductive Biology and Endocrinology*, Vol. 7(89).
- [33] Tubbs, C., Pace, M., Thomas, P. (2010) "Expression and gonadotropin regulation of membrane progesterin receptor alpha in Atlantic croaker (*Micropogonias undulatus*) gonads: Role in gamete maturation" *General and Comparative Endocrinology*, Vol. 165, pp. 144-154.
- [34] Dressing, G.E., Pang, Y., Dong, J., Thomas, P. (2010) "Progesterin Signaling Through mPR $\{\alpha\}$ in Atlantic Croaker Granulosa/Theca Cell Cocultures and Its Involvement in Progesterin Inhibition of Apoptosis" *Endocrinology*, Vol. 151, pp. 5916-5926.
- [35] "Definition of agonist" MedicineNet Accessible at: <http://www.medterms.com/script/main/art.asp?articlekey=7835> (2011-04-29)

- [36] "Receptors as targets for drugs" Accessible at:
<http://www.hcc.bcu.ac.uk/physiology/Receptors2011.htm> (2011-08-10).
- [37] Hai Vu, M.T., Milgrom, E. (1978) "Characterization and assay of the progesterone receptor in rat uterine cytosol" *J. of Endocrinology*, Vol. 76, pp. 21-31.
- [38] Hurd, C., Moudgil, V.K. (1988) "Characterization of R5020 and RU486 Binding to Progesterone Receptor from Calf Uterus" *Biochemistry*, Vol. 27, pp. 3618-3623.
- [39] "Promegestone" Accessible at:
http://www.antialabs.com/product.php?id_product=140 (2011-05-12).
- [40] Mao, J., Regelson, W., Kalimi, M. (1992) "Molecular mechanism of RU 486 action: a review" *Molecular and Cellular Biochemistry*, Vol. 109, pp. 1-8.
- [41] Baird, David. (2009) "Emergency contraception: how does it work?" *Reproductive BioMedicine Online*, Vol. 18 (1), pp. 32-36.
- [42] "RU-486" Accessible at: <http://pubs.acs.org/cen/coverstory/83/8325/8325RU-486.html> (2011-05-12)
- [43] "Functional and Comparative Genomics Fact Sheets" Accessible at:
http://www.ornl.gov/sci/techresources/Human_Genome/faq/compgen.shtml (2011-04-29)
- [44] Silver, M. Lee. (1995) "Mouse Genetics" Accessible at:
<http://www.informatics.jax.org/silver/> (2011-04-19)
- [45] "Centers for Disease Control and Prevention" Accessible at:
http://www.cdc.gov/cancer/nbccedp/cc_basic.htm (2011-04-19)
- [46] Wessel, T.H., Schuchter, U. and Walt, H. (2004) "Ciliary Motility in Bovine Oviducts for Sensing Rapid Non-genomic Reactions upon Exposure to Progesterone" *Horm. Metab. Res*, Vol. 36, pp. 136-141.