Evaluation of Progesterone Receptor Membrane Component 1 as a potential fertility and/or ovulation marker

Sara Ullsten

Degree project, Bachelor of Medical Science, Uppsala University
Kandidatexamsarbete (15 hp) i Biomedicin, Uppsala Universitet
Supervisor: Dr Jens Schuster and Professor Niklas Dahl
# Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
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<tr>
<td>BSA</td>
<td>Bovine Serum Albumin</td>
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<tr>
<td>cDNA</td>
<td>Complementary DNA</td>
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<tr>
<td>DEPC</td>
<td>Diethylpyrocarbonate</td>
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<tr>
<td>DNA</td>
<td>Deoxyribonucleic Acid</td>
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<tr>
<td>EDTA</td>
<td>Ethylene Diamine Tetraacetic Acid</td>
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<tr>
<td>FSH</td>
<td>Follicle-stimulation hormone</td>
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<tr>
<td>GnRH</td>
<td>Gonadotropin-Releasing Hormone</td>
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<td>IVF</td>
<td>In-Vitro Fertilization</td>
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<td>LH</td>
<td>Luteinizing Hormone</td>
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<td>P4</td>
<td>Progesterone</td>
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<tr>
<td>PBS</td>
<td>Phosphate Buffered Saline</td>
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<tr>
<td>PCOS</td>
<td>Polycystic Ovarian Syndrome</td>
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<tr>
<td>PGRMC1</td>
<td>Progesterone Receptor Membrane Component 1</td>
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<tr>
<td>POF</td>
<td>Premature Ovarian Failure</td>
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<td>RNA</td>
<td>Ribonucleic Acid</td>
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<tr>
<td>RPM</td>
<td>Revolutions Per Minute</td>
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<tr>
<td>RT-PCR</td>
<td>Reverse Transcriptase Polymerase Chain Reaction</td>
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<tr>
<td>SDS</td>
<td>Sodium Dodecyl Sulfate</td>
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<tr>
<td>SDS-PAGE</td>
<td>Sodium Dodecyl Sulfate PolyAcrylamid Gel Electrophoresis</td>
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<tr>
<td>TSH</td>
<td>Thyroid-Stimulating Hormone</td>
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<tr>
<td>qPCR</td>
<td>Quantitative Real-Time Polymerase Chain Reaction</td>
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**Introduction**

The menstruation cycle and the ovarian cycle.

The menstrual- and ovarian cycle of approximately 28 days is complex and controlled and synchronized by several coordinating signals from the ovary, brain and pituitary. The participating hormones are gonadotropin-releasing hormone (GnRH) secreted by the hypothalamus, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) both secreted by the anterior pituitary and estrogens and progesterone produced by the ovaries. Alteration and combination of these signals divides the menstrual cycle into three major phases: 1) the menstrual flow phase, 2) proliferative phase and 3) the secretory phase; and the ovarian cycle into two major phases: 1) the follicular phase and 2) the luteal phase. (Campbell and Reece, 2002)

![Diagram showing the menstrual and ovarian cycle phases](http://en.wikipedia.org/wiki/Menstrual_cycle)

The first phase in the menstruation cycle is the menstrual flow phase. During this phase loss of the functional layer of endothelial cells called the endometrium occurs due to shedding of the tissue from the uterus. The endometrium then slowly starts to proliferate and is steadily growing during the one to two week proliferative phase. During the third
and last phase that last for approximately two weeks, the secretory phase, the endometrium continues to proliferate and develops into a more vascularized and gland rich tissue (figure 1). If no embryo has attached to the endometrium wall until the end of the secretory phase a radical drop in hormone levels occurs, resulting a new menstrual flow phase and the cycle is repeated. (Guyton and Hall, 2006; Campbell and Reece, 2002)

The ovarian cycle is parallel with the menstrual cycle and is divided into two phases, the follicular phase and the luteal phase. In between these phases ovulation occurs. In the beginning of the follicular phase several follicles starts to grow in the ovary, inside of each follicle is one egg which slowly grows along with the follicle. As the egg is growing the follicle expands and the coat of follicle cells thickens. Only one of the developing eggs will complete the development while the rest of the follicles disintegrate. At the end of the follicular phase the remaining follicle and the adjacent wall will rupture and release the secondary oocyte – this is called ovulation. After ovulation the remaining follicular tissue will develop into the corpus luteum which secretes female hormones like progesterone during the luteal phase (figure 1). (Rhoades and Bell, 2009; Campbell and Reece, 2002).

**Progesterone**

Progesterone, also known as P4 or the hormone of pregnancy, is a steroid hormone derived from cholesterol (McCance and Huether, 2006). Progesterone and estrogen are together with LH and FSH controlling the female menstruation- and ovarian cycle. It effects the LH and FSH production and affects mammary gland development and mating behaviour (Peluso, 2006). Progesterone effect the pregnancy in several different ways; it maintains the thickened endometrium, thickens the myometrium, relaxes the smooth muscle in the myometrium, prevents lactation until the delivery of the fetus, suppresses the immunological responses to antigens from the fetus and suppresses FSH and LH to prevent another pregnancy, ie, stopping the menstrual cycle (McCance and Huether, 2006; Guyton and Hall, 2006).
It is clear that progesterone has an important role in the ovulatory cycle but its contribution to the follicle and oocyte maturation is not yet clear. It has been discussed that progesterone has an intraovarian site of function effecting granulosa cells and follicular growth (Peluso et al., 2005). Here progesterone inhibits the estrogen secretion from granulosa cells, increasing the progesterone secretion, slows the rate of mitogen-induced mitosis and inhibits granulosa cell apoptosis (Peluso et al., 2006). Recent research has presented a few receptors that may be involved in these intraovarian effects of progesterone. One of these receptors is the non-genomic Progesterone Receptor Membrane Component 1, PGRMC1 (Peluso, 2006).

**Progesterone receptor membrane component 1 (PGRMC1)**

Progesterone Receptor Membrane Component 1 is a 22kDa transmembrane protein expressed in the leukocytes, uterus, adrenal glands, liver and kidney (Schuster et al., 2010). The protein is most often found in the intracellular membranes of the cells but can also be located on the extracellular surface or even in the nucleus (Losel et al., 2008). Its importance is not fully evaluated but the strongest hypothesis is that PGRMC1 binds to steroids like progesterone, even though proof of direct binding has not been shown (Losel et al., 2008). However, recent research showed that PGRMC1 participates in the formation of a multi-protein complex and that this complex can bind different types of ligands. The variety of binding patterns shows that PGRMC1 might have many different functions but changing ligands is not the only way PGRMC1 can vary its function. As the protein is a part of a multi-protein complex it can vary its functions further by forming different kinds of complexes (Losel et al., 2008). For example, PGRMC1 may form a specific complex in the uterus. On the other hand, when expressed in a different tissue along with a different setup of proteins it can establish a different complex with the ability to bind other/additional ligands. Until now it has been showed that PGRMC1-complexes can bind corticosterone, testosterone and cortisol and progesterone with the same affinity (Peluso et al., 2010). PGRMC1 can also bind important non-steroids like heme and cytochrome b5 by its large cytochrome b5/heme-binding domain (Cahill, M.A., 2007) and it has been shown that the protein may positively regulate members of the
cytochrome P450 family which influences intracellular sterol metabolism and steroidogenesis (Huges et al., 2007, Mansouri et al., 2008).

The varieties of PGRMC1 binding options and thus also functions are impressive but further research is needed to elucidate the features of PGRMC1. Indications made from resent studies highlights the probability of PGRMC1 involvement in the ovulatory cycle. One interesting founding is the down-regulation of PGRMC1 in postmenopausal women and women diagnosed with premature ovarian failure and polycystic ovary syndrome. These three conditions are all characterized by loss of or impaired ovulation (Schuster et al., 2010). One explanation of this correlation between PGRMC1 and ovulation is that the protein may mediate important functions of progesterone which is necessary for ovulation. Interestingly, recent studies have shown that if PGRMC1 expression is removed by a RNAi the binding of progesterone to granulosa cells is inhibited and the anti-apoptotic effect of progesterone abolished (Peluso et al, 2008). This has also been shown in cancer studies of PGRMC1 where the results also indicated that the protein is essential for an anti-apoptotic effect in granulosa cells (Peluso, 2006; Peluso et al, 2008).

The importance of PGRMC1 for the survival and development of granulosa cells and thus the follicular growth makes it possible that this protein may be involved in ovarian function and that an alteration in expression or a mutation that changes the structure could impair or alter the fertility in females.

**Infertility**

After two years of trying to conceive a child a couple is considered infertile according to WHO guidelines (WHO 2011) and this diagnose is not uncommon. In Sweden, about 8-9% of all couples are suffering from unwanted childlessness (SCB 2011). The time when a couple is trying to conceive a child without success can be a very stressful and heartbreaking time and most of the patients diagnosed with infertility, especially the females, associates the period of evaluation and treatment of infertility as the most upsetting experience of their life (Freeman, 1985).
The tests that are needed to diagnose infertility and to evaluate each individual case are extensive and time-consuming. The recommended tests are semen analysis for the man and documentation of ovulation, assessment of ovarian reserve, assessment of the uterine cavity and documentation of tubal patency for the woman (Pavone, Hirshfeld-Cytron and Kazer 2011). The female tests are highly dependent on the menstrual cycle and hormone levels which complicate the diagnosis process further. A way to make the first time of infertility investigation easier for the couples would be to make the diagnosing test more effective and faster than it is now. One opportunity to simplify the female diagnostics is to find a parameter to test that do not change during the month and thus can be sampled any day. This opportunity will be discussed in this thesis where a new potential female fertility or ovulation marker is studied.

**Aim**

The aim of the study is to evaluate if PGRMC1 expression/levels can be used as a marker of ovulation and to investigate if PGRMC1 is related to decreased fertility without defined cause.

**Material and methods**

**Patients**

Couples that came to Reproduktionscentrum/Gynekologisk Mottagning at Akademiska Sjukhuset, Uppsala for infertility investigation were asked to participate in this study and after an informed consent was signed a total of 120 women were recruited to the study. Blood samples for PGRMC1 and hormone level analysis were obtained from the subjects after inclusion. The women that were recruited to the study were divided into three main groups after the diagnosed cause of the couples’s infertility. The first group contained women diagnosed with infertility or decreased fertility, this group was later divided into five different subgroups of diagnoses; anovulation, tubular infertility, endometrioses, low ovarian reserve and uterus factor. The second group contained women from couples where the infertility cause was due to the man and the third and last group contained
women where the cause of infertility is unknown. The second group was later used as a control as these women are characterized as healthy.

### Phase separation of blood samples

#### Lysis of erythrocytes

5-10ml of EDTA-blood were mixed with lysis buffer up to a total volume of 45 ml. The samples was incubated on ice for 15 minutes to allow the erythrocytes to lyse and then centrifuged for 15 minutes at 2200 rpm. The pellet was resuspended in 15 ml lysation buffer and then centrifuged for 15 minutes at 2200 rpm. The formed pellet contains nucleated leukocytes. The pellet was resuspended in 1 ml TRIZOL which disrupts the integrity of the cells and dissolve cell components but retains RNA integrity. The total TRIZOL and cell lysate was then frozen at -20°C until protein isolation was continued.

#### RNA and protein separation

Protein was precipitated with TRIZOL (Invitrogen) from EDTA-blood samples according to manufacturers’ protocol. TRIZOL or Guanidinium thiocyanate-phenol-chloroform extraction as the method is named was developed by Chomczynski and Sacchi (Chomczynski and Sacchi, 1987) and is a commonly used technique to isolate RNA, DNA and proteins from various types of tissues and cell cultures. 0.2ml of chloroform was added to 1ml Trizol/cell lysate sample which divides the mixture into an aquatic, inter and organic phase. RNA will remain in the aquatic phase, DNA in the inter phase and proteins will remain in the phenol-chloroform phase. The aquatic phase, containing RNA, was removed and mixed with 0.5ml isopropyl alcohol, inverted and incubated in room temperature for 10 minutes. After centrifugation at 12 000 x g for 10 minutes at 2-8°C the RNA precipitate formed a gel-like pellet. The pellet was washed by vortexing in 70% ethanol and redissolved in 50µl DEPC-treated water. Final RNA concentration and quality was measured with a spectrophotometer (Nano-Drop, Saveen Werner).

The remaining inter- and phenol-chloroform phase was mixed with ethanol to precipitate the DNA in the interphase. After inversion and incubation for 2-3 minutes in room temperature the samples was centrifuged to sediment the DNA. The supernatant was transferred to a new Eppendorf tube. For a final precipitation of proteins from the
organic phase isopropyl alcohol was added and the protein pellet was washed 3 times in 0.3M Guanidine hydrochloride in 95% ethanol. As a final wash the pellet was vortexed in ethanol and then dissolved in 1% SDS at 50°C overnight on shaker. The precipitated RNA and DNA samples were stored at -20°C.

**Western Blot**

Western Blot is a commonly used method to detect proteins using specific antibodies. The method is divided in two steps, separation and detection. Separation of protein samples was performed by 12% SDS-polyacrylamide gel electrophoresis (SDS-PAGE, NuPAGE, Invitrogen) for 1 hour at 180V in 1X SDS Running Buffer (NuPAGE, Invitrogen). 250µl of Antioxidant (NuPAGE, Invitrogen) was added between the gels before the run. 1µl prestained protein ladder (Fermentas) was used to detect protein size. 15µl sample were mixed with 5µl 4X Loading Buffer (NuPAGE, Invitrogen) and 2µl 10X Reducing agent (NuPAGE, Invitrogen), incubated for 10 minutes at 70+ C° and then loaded on the gel. Following separation of denatured proteins, proteins were transferred to a PDVF membrane by iBLOT® Dry Blotting system (Invitrogen) and afterwards the membrane was placed in PBS. Membranes were incubated overnight at 4-8°C sequentially with primary antibodies for PGMRC1 (1:500, produced in rabbit, Invitrogen) and β-actin (1:10 000, produced in mouse, Abcam) diluted in 1 or 5% BSA in PBS, respectively. Subsequently membranes were incubated for one hour at room temperature with IRD680- or IRD800 labeled secondary antibodies (α-mouse and α-rabbit,1:20 000, produced in donkey, LiCor Biosciences), diluted in 1 or 5% BSA in PBS. Following each incubation, membranes were washed three times in 0.1% tween in PBS for at least 7 minutes. An additional washing step in PBS was performed before membrane scanning by Odyssey infrared imaging system. Integrated intensity of β-actin and PGRMC1 bands were obtained by Odyssey 2.1 software (LiCor BioSciences).

**RNA integrity**

To investigate the quality of isolated RNA samples were analyzed by gel electrophoresis with Agilent RNA 6000 Nano Kit (Agilent Technologies). RNA 6000 Nano Marker, Agilent RNA 6000 ladder, RNA Nano dye concentrate and Agilent RNA 6000 Nano Gel
Matrix were included in the kit and used following manufacturer’s instructions. 1 µl of each selected sample was added to the chip and the chip was inserted in the Agilent 2100 Bioanalyzer and analyzed by Agilent 2100 expert software.

**cDNA Synthesis**

After RNA isolation first strand cDNA was synthesized by Reverse Transcriptase PCR (RT-PCR) using the RevertAid™ H Minus First Strand cDNA Synthesis Kit (Fermentas). 1 µg RNA was used in each reaction and mixed with Random Hexamer Primers for a final primer concentration of 1.0 µM. DEPC-treated water was added for a total volume of 14 µl. The mixture was incubated for 5 minutes at 70°C for denaturation. 4 µl MMLV buffer (5X), 1 µl Ribonuclease Inhibitor (RiboLock™ RNase Inhibitor, Fermentas) and 1 mM of each nucleotide was added to the samples, followed by an incubation for 5 minutes at 25°C. The enzyme Reverse Transcriptase was added last and thereafter the samples were first incubated at 25°C for 10 minutes and followed by incubation at 42°C for 60 minutes. During this time the enzyme synthesizes first strand cDNA. To inactivate the enzyme, samples were heated to 70°C for 10 minutes. All reagents except RNA and nucleotides were included in the kit and all steps except incubations were performed on ice.

**qPCR**

Quantitative Real-Time PCR (qPCR) was performed on synthesized cDNA using Platinum® SYBR® Green qPCR SuperMix UDG (Invitrogen). SYBR green is an asymmetrical cyanine dye that nonspecifically binds double stranded DNA. Formation of the dye-DNA complex will cause florescence which can be measured by qPCR equipment. As the product increases after each PCR cycle the fluorescence will also increase in intensity, thus make it possible to quantify DNA concentration after each cycle in real time. The ready to use 2X SuperMix contains all reagents for qPCR except cDNA and primers. Forward and reverse primers (sequence in table 1) for the gene of interest, PGRMC1, and the housekeeping gene β-actin were added for a final concentration of 2 µM, respectively. The first strand cDNA was diluted 1:20 after synthesis and added to the qPCR reaction. Water was added for a total sample volume of
20µl. The qPCR reaction (see table 2) was performed on a Stratagene® Mx3005P (AH diagnostics) and the results analyzed by the MxPro software.

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<th>Gene</th>
<th>Fw/Rev</th>
<th>Primer sequence</th>
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<tr>
<td>B-actin</td>
<td>Fw</td>
<td>5’-CTGGCAACGCTGGAAGGTGACA</td>
</tr>
<tr>
<td></td>
<td>Rev</td>
<td>5’-CGGCCACATTGTGAACCTTTG</td>
</tr>
<tr>
<td>PGRMC1</td>
<td>Fw</td>
<td>5’-GGGTGTTCGATGTGACCAAAG</td>
</tr>
<tr>
<td></td>
<td>Rev</td>
<td>5’-GATGACATCTCTTCAGCAAT</td>
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Table 1. Primer sequence used in qPCR

<table>
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<tr>
<th>Temp (°C)</th>
<th>Time</th>
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<tr>
<td>50</td>
<td>2 min</td>
</tr>
<tr>
<td>95</td>
<td>2 min</td>
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<tr>
<td>95</td>
<td>15 sec</td>
</tr>
<tr>
<td>60</td>
<td>60 sec</td>
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\{ 45 cycles \}

Melting curve analysis

Table 2. qPCR reaction schedule performed by Stratagene® Mx3005P (AH diagnostics)

Statistics

Data was analyzed by statistical test to evaluate the distribution of single data points (Kolmogorov-Smirnov normality test). Normal distributed data was further analyzed by ANOVA-test to compare variance of averages of several groups. Subsequently, independent pairwise t-test were performed where p<0.05 was considered as significant.

Results

To investigate whether PGRMC1 can be used as a fertility and/or ovulation marker the RNA levels and protein expression of PGRMC1 was evaluated. From the 120 women that were allocated to the study RNA and proteins were isolated from EDTA-blood samples. The isolated proteins were separated by a SDS-PAGE gel electrophoresis and were analyzed by Western blot. A normalized integrated PGRMC1 value was obtained by normalizing the value by the internal control integrated β-actin. The quality of isolated RNA by an Agilent RNA 6000 Nano kit and the amount of RNA was thereafter measured by Nano-Drop. First strand cDNA was subsequently produced by RT-PCR and the cDNA
product was analyzed by qPCR. The expression of the PGRMC1 gene was normalized by the housekeeping gene β-actin as were used in the protein analyze. All data were statistically analyzed by Kolmogorov-Smirnov normality test to evaluate the distribution of the data and normal distributed data was subsequently analyzed by ANOVA test and independent pairwise t-test to identify differences of sample means.

**Protein**

All samples were analyzed at twice by Western Blot, example blot is seen in figure 2. The different samples were loaded randomly to the different wells to rule out possible errors that can occur in the outer wells due to running artifacts. Two values where calculated from each sample, integrated β-actin intensity and integrated PGRMC1 intensity by Odyssey software. β-actin was used as an internal control to minimize errors due to differences in blood sample preparations and protein purification. To obtain a normalized PGRMC1 level for each run the integrated PGRMC1 intensity was divided by the integrated β-actin intensity. Variations where seen both between samples and within samples. To minimize Western Blot errors an average PGRMC1 level was calculated from the two duplicates. This value was used for comparison between samples and statistical analysis. The PGRMC1 levels were grouped according to diagnosis given by personnel at Reproduktionscentrum/Gynekologisk Mottagning at Akademiska Sjukhuset, Uppsala. The results were organized in a box plot with whiskers and are presented in figure 3. The upper point of the higher line shows the highest value, the lowest point on the lower line shows the smallest value. The box in between the lines shows the $3^{rd}$ quartile to the $1^{st}$ quartile and the cross in the middle marks the median. A statistical analysis was performed on the data which shows that the data is normally distributed. I used an ANOVA (analysis of variance) to detect any differences in PGRMC1 expression between the groups, but no significant difference could be detected.
Figure 2. Western blot analysis of PGRMC1 protein expression. Western blot membrane after scanning by Odyssey infrared imaging system. The first well to the left is the protein ladder and the other four wells are samples. The first bright red band in each sample is the internal control β-actin and the third band that lies in between two green bands is PGRMC1.

Figure 3. Analysis of PGRMC1 expression in women that are suffering from unwanted childlessness. The box plot is showing average PGRMC1 protein values calculated from two duplicate analyzes of each sample. The Y-axis is the normalized PGRMC1 protein level with and the X-axis is the different diagnose groups. All women included in the study were allocated to one diagnose after investigation at Reproduktionscentrum/Gynekologisk Mottagning at Akademiska Sjukhuset, Uppsala.

RNA

RNA was isolated from EDTA-blood samples. And the purity and amount of RNA was measured by spectrophotometer (Nano-Drop, Saveen Werner) Subsequently, RNA integrity/quality was tested on a set of random samples by RNA chip (Figure 4). Some samples had a “smear” in the column which can be interpreted as poor RNA integrity. This could be neglected as two distinct bands, the 18S and 28S subunits, could be seen in all analyzed samples which indicate a successful RNA purification.
Figure 4. RNA chip outcome. Random samples where analyzed to investigate RNA integrity. The first column with six distinct bands is a RNA ladder and the following samples contains isolated RNA from patients. Two clear bands (18S and 28S subunits) indicate a sample with good integrity. Samples with two bands and a “smear” (sample 1 and 7) indicate degraded RNA.

First strand cDNA was produced by RT-PCR and was used in qPCR analysis to analyze the RNA expression of the PGRMC1 gene. The cDNA samples were run in triplicates in two different qPCR runs to minimize experimental errors. The results were grouped into the same diagnosis groups as the Western Blot results and are presented as a box plot (figure 5). The statistical analysis of the data (performed as above) showed that the values were normally distributed and no significant difference between the groups could be found.

Figure 5. Analysis of PGRMC1 RNA expression in women suffering from unwanted childlessness. The box plot is showing average PGRMC1 RNA levels. The Y-axis is the RNA levels in comparison to the housekeeping gene β-actin and the X-axis is the different diagnose groups. All women included in the study was allocated to one diagnose after investigation at Reproduktionscentrum/Gynekologisk Mottagning at Akademiska Sjukhuset, Uppsala.
Hormones
Together with diagnosis levels of hormones like FSH, TSH and prolacin was given from Reproduktionscentrum/Gynekologisk Mottagning at Akademiska Sjukhuset, Uppsala. These hormone levels were grouped according to diagnosis as both protein and RNA levels have been grouped (see figure 6). The three hormone are relatively stable and do not vary a lot between the different groups and a large intra sample variation can be seen. There are two groups that are slightly increased in regard to their FSH levels. These groups are the one with tubular infertility diagnosis and the group diagnosed with endometriosis. In these two groups the number of samples is low (tubular infertility 3 samples and endometriosis 1 sample) which should be considered during analysis.

Figure 6. Analysis of hormone levels in women suffering from unwanted childlessness. The box plot is showing hormone levels grouped after each diagnose. These values were obtained by personnel at Reproduktionscentrum / Gynekologisk Mottagning at Akademiska Sjukhuset, Uppsala.
Discussion

In this thesis the levels of the transmembrane protein PGRMC1 and its RNA expression have been investigated in females that are suffering from unwanted childlessness. The aim was to detect significant differences between healthy women and women diagnosed with decreased fertility which would enable PGRMC1 to be used as a marker of fertility and/or ovulation.

Unfortunately no significant differences, in either protein or RNA expression, were found between the different diagnosis groups of women. However, large variations within the samples, most importantly in the Western Blot analysis, could be one reason why the data is widely spread and thereby no significant difference could be seen. To decrease the difference within the samples they could be analyzed more than two times or a standard control sample could be used one each blot which the other samples could be related to by setting this one to 1. Each of these improvements would give a more secure value for each individual and decrease the large standard deviation in each group. If the samples still show this large variation after extended analysis the accuracy of the method most be evaluated and maybe exchanged to a more robust method.

Previous studies of PGRMC1 levels have shown differences in protein levels in women with certain diseases but no differences in RNA levels (unpublished data). This indicates that the RNA levels and protein levels are not correlated and some kind of translational regulation applies. However, this assumption contradicts some patterns that can be seen in this study. Even though no significant difference can be seen in either analysis similar patterns can be seen when the two methods are compared. Tendency of similarity can be seen when comparing RNA and protein values of patients suffering from tubular infertility and endometriosis. The patients diagnosed with tubular infertility tend to show higher RNA and protein levels than the other groups and patients diagnosed with endometriosis tend to have lower values. Unfortunately no significantly difference can be find between these groups and the others, perhaps due to a low sample size, but these results are interesting to remember for later analysis.
Earlier studies have not shown any correlation between PGRMC1 levels and different hormones associated with ovulation and pregnancy (unpublished data). These data corresponds to the ones presented in this thesis. It seems like the hormone levels are similar in the different groups which indicates that the hormones are not involved in the disease outcome. Only two groups found in the FSH box plot seem to differ from the others, these two groups are the tubular infertility group and the groups diagnosed with endometriosis. In both groups the sample value is low (tubular infertility 3 samples and endometriosis 1 sample) and thus a valid conclusion is hard to give. When more samples have been analyzed these two groups would be interesting to analyze to see if there is a significant difference in FSH levels.

**Conclusion**

No significant difference have been seen between the groups that been analyzed in this thesis. It is important to remember that a large intra sample variation have been seen during this study and before any certain conclusions about PGRMC1 and its capability to be used as an fertility or ovulation marker can be made the method must be improved. The first step would be to run the samples more times in the Western Blot analysis and to relate all samples to a common control sample to get a better and more accurate mean value. If this is hard to conceive and the intra sample values are still varying another method should be considered.
References


Schuster, J. et al., 2010. Down-regulation of progesterone receptor membrane component 1 (PGRMC1) in peripheral nucleated blood cells associated with premature ovarian failure (POF) and polycystic ovary syndrome (PCOS). *Reproductive Biology and Endocrinology*, 8:58
<http://www.who.int/topics/infertility/en> [assessed august 23 2011]