THERMODYNAMIC ASPECTS OF AGE-DEPENDENT DETERIORATION OF MYOMETRIUM AND DETRUSOR MUSCLES

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THERMODYNAMIC ASPECTS OF AGE-DEPENDENT DETERIORATION OF MYOMETRIUM AND DETRUSOR MUSCLES

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ABSTRACT

THERMODYNAMIC ASPECTS OF AGE-DEPENDENT DETERIORATION OF MYOMETRIUM AND DETRUSOR MUSCLES

Problems in labor and delivery effects millions of people every year and are the reason for a high number of mortality around the world. Due to the changing social increased age when giving birth is observed which has made treating older females that have difficulty when conceiving and giving birth an important part of today's medicine. In pregnancy, the myometrium turns from silent to and actively contracting organ to provide a decent delivery of the baby. Oxytocin is produced in the uterus and its application can be used to induce uterine contractions when giving birth. It is an effective stimulator and it can increase the frequency, force and the duration of the contractions. Due to the increase of the population of elderly people in developed countries will result in higher number of people dealing with problems related to bladder dysfunctions. Due to aging, micturition dynamics in vivo and contractile strength in vitro can have different responses to neurotransmitters. Isolated tissue baths are a classic apparatus that helps in determining the concentration-response relations in various tissues. It has been around over 150 years and due to its versatility and simplicity, it has been an important device for the scientists. In this study, organ bath method was used to measure the effects of aging on myometrium and detrusor tissues.

ÖZET

MYOMETRIUM VE DETRUSOR KASLARININ BOZUNMASININ YAŞA GÖRE DEĞİŞİMİNİN TERMODİNAMİK YÖNLERİ

Doğum sırasında yaşanan problemler, her yıl milyonlarca kişiyi etkilemekte ve ölüm oranlarında yüksek bir payı bulunmaktadır. Değişen sosyal trendler sonucu, ilerleyen yaşlarda da doğum yapıldığı görülmekte ve bu sebeple günümüz tıbbında hamile kalmak ve doğum sırasında yaşlı kadınlarında yaşadığı sıkıntıları çözmek önem kazanmaktadır. Doğum sırasında rahim sessiz bir organdan, aktif olarak kasılan bir organa dönüşerek bebeğin doğumunda yer alır. Oksitosin kasılmaların sıklığını, gücünü ve süresini arttıran bir kimyasal uyarıcıdır ve doğum sırasında kullanılabilmektedir. Artan yaşlı nüfus dolayısıyla gelişmiş ülkelerde yaşanan mesane sorunları da artmaktadır. Yaşlanma ile beraber idrar çıkarma sıklığında yaşanan sorunlar da akmaktadır ve yaşa bağlı olarak farklı uyarıcılara verilen kasılma tepkileri de farklı olabilir. Organ banyosu yüz yıllardır kullanılan ve konsantrasyon-tepki ilişkini bir çok dokuda incelemeye sağlayan bir metottur. 150 yıldır olan bu metot, değişkenliği ve kolaylığı dolayısıyla, doku çalışmaları yapan bilim insanları için önemli bir alet olmuştur. Bu çalışmada organ banyosu metodu kullanılarak rahim ve mesane kaslarının yaşa göre kasılmasında olan değişiklikler incelenmiştir.

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LIST OF SYMBOLS/ABBREVIATIONS

CRH	Corticotrophin releasing hormone
FSH	Follicle stimulating hormone
LH	Luteinizing hormone
MLC	Myosin light chain
OAB	Over active bladder
OT	Oxytocin
OTR	Oxytocin receptors

1. INTRODUCTION

Issues in labor and delivery effects millions of people every year and they are responsible for a good number of mortality around the world. Improper contractions of the uterine muscle can lead to many issues such as dystocia, uterine atony and post-partum hemorrhage [1,2].

The changing social trends that results in giving importance to career caused increased age when giving birth which has made treating older females that have difficulty when conceiving and giving birth an important part of today's medicine [3]. Fertility of females starts decreasing around their mid-30s both due to the decline in the conception rate and increase in pregnancy loss because of aneuploidy [4,5]. Increasing age results in depletion of ovarian follicles and decrease in oocyte stability which causes aneuploidy. Reproductivity in females includes individual variability but in general, reproductive senescence is observed in ages 40 to 60. Environmental and genetic factors may be considered the reasons for the varying reproductive senescence due to their effect on cellular aging [4].

Population of elderly people in developed countries is increasing and it will continue in the future and as a result more people will have to deal with problems related to bladder dysfunctions [6]. About 10 percent of the elderly population is affected by the urinary incontinence caused by the abnormal spontaneous contraction of the bladder muscles and treatments for this have not been completely optimized [6].

1.1. MUSCLE CELLS

Muscles in vertebrates can be classified as three classes, skeletal muscles, smooth muscles and cardiac muscles. Skeletal muscles connect the bones in the body whereas smooth muscles surround internal organ like blood vessels and the uterus [7]. Cardiac muscles on the other hand, are responsible for spontaneous movements such as heart pumping [8]. Contraction of these muscles depends the formation of actin-myosin complex which is an ATP dependent process [7,8]. Smooth muscle cells have the ability to contract and relax slowly, and generate and retain tension longer than skeletal muscles [7]. They are more

dependent on the extracellular calcium ion while skeletal muscles are more resistant to its removal [9]. Contraction of uterine smooth muscles are affected by hormonal, neuronal, metabolic and mechanical stimulation [10,11].

1.1.1. Contractile Mechanism of Smooth Muscles

Smooth muscles can be found in several parts in the body such as stomach, intestines, bladder, airways, uterus and other organs. Smooth muscles cells do not have the striated banding pattern like the cardiac or skeletal muscles. Contraction of the smooth muscles is governed by the hormones, autocrine/paracrine agents and various chemical signals. Indifferent to the stimulus, cross-bridges between actin and myosin generates forces and contraction is initiated by the Ca^{2+} [12].

The initiated contraction in smooth muscles by Ca^{2+} occurs in thick filaments and in striated muscles it occurs in the thin filaments. When stimulated, intracellular concentration of calcium ion increases and it couples with calmodulin and as a result, myosin light chain (MLC) kinase is activated which in turn phosphorylate the light chain of myosin. The release of calcium ion from the sarcoplasmic reticulum and the passing of ions from the extracellular matrix causes an increase of cytosolic calcium ion. MLC kinase activation can also occur due the regulation of MLC phosphatase. When the myosin binding subunit is phosphorylated, the enzymatic activity of MLC phosphatase is prevented and contraction occurs [12].

Uterine smooth muscle, myometrium, is made up of a muscular layer of uterus that causes dilation of the cervix and delivering of the fetus as a result of contractions. These contractions does not require hormonal or nervous input, they occur spontaneously. The casual depolarization of the myometrial cell causes an opening of the voltage operated calcium channels and thus an influx of calcium into the cell which in turn causes contractions. Calmodulin, along with the calcium complexes activates the MLC which then phosphorylates myosin enabling cross bridge cycle formation [13].

Relaxation of smooth muscles occurs when the stimulus is removed or when a substance inhibits contractile mechanism. Either way, relaxation occurs when the calcium ion concentration declines and MLC phosphatase activity is increased. In the presence of MLC

phosphatase, the high energy phosphate from the light chain is pulled out of light chain of myosin and muscle relaxation is stimulated [12].

1.2. HUMAN PARTURITION

Labor is a complex process and many regulatory pathways take place in a harmony for it initiation. Timing of this is crucial as it affects the fetal development significantly. A full-term pregnancy is considered to occur at 39 weeks, but 37 and 42 weeks, it is considered early and late term pregnancy respectively [2]. After 42 weeks, it is deemed overdue and in 5 to 15 percent of the pregnancies, preterm birth may occur [2,14].

The same biochemical features that govern contractility is present in the uterus myometrium as those of the smooth muscles [15]. Uterus is among the spontaneously active smooth muscle groups meaning that, even without any stimulation and unrelated to being pregnant or not, it will generate spontaneous contractions [16]. Activity of the uterine muscles and their function depends on the estrogen and progesterone levels. High estrogen levels results in high amplitude of uterine contraction whereas progesterone decreases the occurrence and tension of the contractions due to progesterone's function of downregulating prostaglandins, oxytocin receptors and calcium channels [17]. Uterus muscles can also be stimulated by the stretch and in the contraction can occur [16].

Estrogen is crucial for development and function of uterine and it has significant roles in uterine contractility as it synthesizes contractile proteins and the regulatory enzymes. In females, uterus starts growing after secretion of estrogen following the puberty and the uterus gains the capability of responding to stimulants and inhibitors of contractions. Estrogen is critical for intracellular communication and it raises the receptor concentration for oxytocin that in turn modulates membrane calcium channels [18].

At the early stages of the pregnancy, main source of progesterone is corpus luteum and its removal may lead to losing the pregnancy. It has been shown, in vitro, that myometrial contractility decrease is related to progesterone. During pregnancy, estrogen and progesterone is in a harmony which controls the uterine activity. It can be said that progesterone is an important factor in uterine quiescence and cervical integrity as it takes

place in development of calcium channels and oxytocin receptors which are involved in contraction of myometrium [18].

Oxytocin is produced in the uterus and its infusion can be used to induce uterine contractions an labor [18]. It is an effective stimulator and it can increase the frequency, force and the duration of the contractions and it takes place in the initiation and progress of the labor [16,19]. During the labor process, it takes place in expulsion of placenta and prevents hemorrhage [16]. Oxytocin receptor expression increases with estrogen which occurs into to the end of pregnancy. The contraction by oxytocin occurs through the stimulation of prostaglandins E_2 and prostaglandin F2α release by phospholipase C activation, as well as directly by phospholipase C which activates calcium channels and releases calcium from intercellular stores [18]. Although the contractile mechanisms are caused by Ca^{2+}, the oxytocin can also result in Ca-independent contraction although is it only about 5-15 percent when compared to those obtained with calcium [12,16].

1.2.1. Myometrium Contraction

Myometrium is the middle layer of the uterine wall. In the uterus inner layer is called the endometrium and the perimetrium is the outer later. Myometrium is made up of smooth muscle cells and has the typical features that are known for the smooth muscles such as the slow but strong contractions with an extensive movement range that is needed during the labor [2]. But its role is not just limited labor. In labor myometrium contraction time and its amount of contraction is critical as it serves for the delivering of the baby. In other phases of a women's life, it keeps toned and remain relaxed in non-pregnant and non-menstruating periods. Proper contraction of the muscle is important because the presence of undesired contraction of myometrium may result in abortions or delivery before term and the extreme contraction of the muscle can cause distress in the fetus, hypoxia and sometimes may even lead to loss of the baby. Also if the contractions are not sufficient or irregular, it may require the babies to be delivered via c-section [20].

Non-pregnant uterus myometrium has different types of contraction in various phases of the cycle. They are wave like rhythmic contractions which is also known as uterine peristalsis and focal and sporadic bulging of the myometrium helping with the continuous contractions. These contractions aid in passage of sperm in the canal and shedding of the endometrium

[11,20]. In pregnancy, the myometrium turns from silent to and actively contracting organ to provide a decent delivery of the baby. This is accomplished by the interaction of estrogens and progesterone, with the balance between these compounds. Nonetheless, main reason for the contraction of the uteri is the contractile activity of the cellular element, the uterine myocytes. The uterine myocytes is mainly regulated by the intercellular Ca2+ concentration [20].

1.2.2. Process of the Parturition

The timing of the birth in humans is related with the placenta development which works in the expression of a gene for the corticotrophin releasing hormone (CRH). As pregnancy progresses, the plasma levels of CRH increase and it peaks at the delivering of the baby. This placental CRH production, also changes with the effect of estrogen, progesterone and nitric oxide in an inhibitory way while neuropeptides work as a stimulant for this hormone. It has also been reported that CRH increases the effect of some uterotonics such as oxytocin and prostaglandin which work in the contraction of the uterus [14].

In labor, expression of contraction associated proteins are another highly important event, because acting in the uterus they aid in starting of the powerful and rhythmic contractions that causes the passage of the fetus when in term. These proteins have three different types. One is responsible for enhancing the interaction between the actin and myosin proteins to result in contraction, other increases the excitability of the myometrial cells and the other one boost the intercellular connectivity that allows for a synchronous contractions [14].

During labor the myometrium turns from a tissue with low connectivity between the myocytes to a tissue with high level of physical connections which happens through the pores made by the multimers of connexin 43. These connection between individual myocytes also occurs through the paracrine release of prostaglandin $F_{2\alpha}$ and the release of calcium. This way, myocytes are depolarized that can be passed to a neighbor cell and this way powerful waves of depolarization and contraction occurs in the uterus and through the increase intrauterine pressure, fetus can be expelled [14].

Uterine myocytes have a high number of myofilaments [21]. The contractility of the myocytes occur due to the interactions between the actin and myosin. These interaction takes

place the actin is converted from a globular form to a filamentous form. Myosin on the other hand is activated when phosphorylation occur via myosin light-chain kinase which is activated by the calmodulin and internal calcium in the cells. After the depolarization of the myocyte, and influx of extracellular calcium goes through the channels and calcium is released from the intracellular stores. This results in an increase in the intracellular calcium that encourages the actin and myosin interaction and in turn, this causes contraction [14].

An important element of the myometrial activity is the synchronization of the cells which is need for the expulsion of the fetus through powerful contractions. Another element that is considered important is the relaxation of during these contraction. During contraction, blood flow to the fetus decreases and via relaxation, blood flow to the fetus is allowed. The synchronization between these events occur with the electrical conduction through connecting myofibrils that conducts the electrical activity to adjacent muscle fibers and with the activated myocytes, production of prostaglandins works in depolarizing neighboring myocytes. As more myocytes join the activity with this process, they work together in contraction followed by the relaxation. A regular uterine contraction may take about a minute and it is consisted of a slow rise and fall of the tension [14].

Prior to labor, these myocytes stays at a high interior electronegativity which decreases the possibility of depolarization and contraction. ATPase driven sodium-potassium pump helps create a resting membrane potential and through the potassium channels, potassium can leave the cell and increases the intracellular electronegativity and as the potassium channels are kept open via myometrial cell surface receptors, the muscle can stay at a relax stage. During labor, when oxytocin and prostaglandin $F_{2\alpha}$ binds to the receptors on the surface of the cell, ligand regulated calcium channels are opened. Penetration of the calcium into the cell causes a decrease in the electronegativity and causes the voltage regulated calcium channels to be opened and depolarization occurs with the swift movement of these ions into the cell [14].

Before the labor, these myocytes are kept at a relaxed state through the increasing of the intracellular cyclic AMP (cAMP). When cAmp increased, it activates protein kinase A and it works in dephosphorylation of myosin light chain kinase and increases phosphodiesterase activity. The process of the relaxation is also developed by the mechanism that keeps the actin in globular form and hindering the actin fibril formation that is need for contraction to occur. When the time for labor comes, these mechanisms are reversed. In the myocytes, actin

turns into fibrillar form and through the entry of calcium into the depolarizing cell, calmodulin-calcium complexes occur that in turn activates the myosin light chain kinase that in return phosphorylates the myosin light chain. This phosphorylation results in the ATPase activity generation and causes the sliding of myosin over the actin filament that results in the contraction [14].

1.2.3. Oxytocin Mechanism

Oxytocin (OT) has many roles in the pathological and physiological process from reproduction to maternal behavior. It can affect the social behavior as it increases empathy, creates trust and pair bonding. It is synthesized by the pituitary gland, peripheral tissues such as uterus lining, corpus luteum as well as placenta and has a role in effecting energy balances as well through several mechanism [2,22,23]. In the parturition process, progesterone, estrogen and oxytocin is widely involved [21]. It was the first peptide hormone to have its structure discovered and its was also the first to be synthesized in an biologically active form and consists of nine amino acids [2,23]. OT can take place in stimulation of the contraction of the myometrium [23]. During labor process, due to Ferguson reflex, oxytocin is secreted and a positive feedback loop takes place where the uterus applies pressure to cervix that causes a stimulation that causes the secretion of oxytocin from the pituitary gland. Afterwards, frequency and force of the contraction enhances which then increases the signaling of the gland. From there on, more force and frequency is observed during the contractions [2].

In many placental mammals along with humans, increase in plasma oxytocin levels has been observed at some stages during parturition [22]. It has been suggested that when the estrogen to progesterone ratio increases prior to beginning of the labor which then causes higher level of oxytocin receptors [21]. But due to technical difficulties exact levels of oxytocin in labor is hard to measure as it has a short half-life and it experiences fast metabolism. As it is produced locally by the intrauterine tissues, their closeness to the myometrium shows that paracrine oxytocin (OT) signaling system in these intrauterine tissues, can be partly responsible of the labor onset and progression. Nonetheless, the circulating oxytocin may not be fundamental in labor as labor in human may also happen when maternal pituitary gland dysfunction. Also, in a study with oxytocin deficient mice have also been reported to

deliver in a regular way, suggesting that other pathways working in parturition may be making up for the loss of oxytocin [22].

In myometrial tissues of humans, increase in oxytocin receptors (OTR) and their amount and increase in OTR mRNA levels during beginning of the labor happens to intervene in the increased sensitivity of myometrium to oxytocin. In beginning of the pregnancy, OTR's are expressed in low amounts but by the 37-41 weeks, they can rise by twelve fold and they are present at maximum amount after the starting of the labor [22].

Oxytocin can also be used to induce labor by causing uterine contraction before the spontaneous starting of the labor [1,21,22]. This is mainly performed when the early delivery of the baby is more advantageous than continuing the pregnancy. Other than its role as an inducing agent, it also has a role in improving the labor. It has been estimated that around 20-30 percent of women receives induction of labor and two thirds of these women can give birth to their babies without additional interference while other may need c-section [22].

There are some studies that proposes that use of intravenous oxytocin only on its own is not ideal for induction of the labor because in comparison to vaginal PGE_2 as an inducing agent, use of oxytocin on its own can cause less vaginal deliveries within 24 hours, a lower Bishop score which is a method to determine cervical favorability and results in higher number of c-section. Thus application of oxytocin is mainly performed after an initial treatment with prostaglandin when enough cervical opening is observed [22].

1.3. RAT REPRODUCTIVE CYCLE

Laboratory rats are commonly used in many studies, such as behavioral, genetic or nutritional studies. They are also used in experiments to model mammalian health and diseases and they have made various contributions to reproductive researches [24]. But since there are some differences between humans and rats, it is important that the experiments take into consideration these variances [1].

The reproductive system in rats includes a genital tract and two ovaries. Oviducts, cervix, vagina and uterus makes up the overall genital tract. Two separated uterus horns are present in the uterus and this way, rats are able to have several off springs [24]. However in humans, these shape of the uterus has a pyriform, which is a light bulb shape, whereas in rats, it

consists of two-horned uteri with several implantation site in both of the horns as seen in Table 1.1. [1].

Table 1.1. Comparison of uterine anatomy and histology, off spring number and length of gestation in rats and humans [1]

	Rat	**Human**
Uterine anatomy	Two horned	Pyriform
Uterine histology	Defined muscle layers	Mixed muscle layers
Off spring number	5-12 pups	1-2 children
Gestation length	22-23 days	259-294 days

Puberty of female rats begin around four weeks after being born when the luteinizing hormone (LH) is produced which leads to ovarian maturation. The initial period where the changes in LH release starts is called anestrus which happens around eight days before the proestrus [25].

In sexually matured rats, reproductive cycle is called an estrous cycle [26]. This cycle occurs all year round and seasons do not have an effect on it. It starts about 33 to 42 days after being born [24]. The estrous cycle consists of four phases called proestrus, estrus, metestrus and diestrus which happen consecutively and lasts around four to five days and repeats five to six times a month [26]. This may increase as the rats ages and may last around 6 days by the end of the reproductive life span [24].

The stages in the estrous cycle varies in time as shown in Table 1.2. Proestrus, estrus, metestrus and diestrus takes around 12, 9-15, 21 and over 57 hours respectively, diestrus being the longest one. In the cycle, hormones have important roles. Anterior pituitary secretes gonadotrophins and these help in the regulation of the estrus cycle with the luteinizing hormone (LH) and follicle stimulating hormone (FSH). The follicle growth is stimulated by the FSH and the follicles to ovulate and making up of the corpus luteum happens with the LH. During metestrus, corpus luteum secretes progesterone, but its levels decrease during the diestrus phase. Estradiol 17β increases along the follicular development and the end of the cycle is observed as estrogen peak occur in the proestrus, and gonadotropin release is stimulated to start ovulation [24].

Table 1.2. Morphology of the vaginal smears and behavior of the rat [24]

Phase	Duration (hour)	Behavior of the rat	Vaginal cytology
Proestrus	12	Acceptance of male by the end	Nucleated epithelial cells
Estrus	12	Male acceptance	25% cornified cells and 75% nucleated cells
Metestrus	21	No male acceptance	Leukocytes, nucleated and cornified cells
Diestrus	51	No male acceptance	Leukocytes

Determination of the phases is includes examination of the vaginal cytology and changes in the behavior. Vaginal cytology examination is used commonly as it is a rapid and useful method. In order to identify the phases, smears are taken at settled times every day. This is because cell populations changes in a 24 hour period. Proestrus and estrus are the only phases that the rat is ready to accept males at the end of the proestrus phase and vaginal cytology is made up of nucleated epithelial cells. In estrus, both nucleated and cornified cells are observed and in metestrus leukocytes, nucleated and cornified cells are observed. In diestrus only leukocytes are observed and it is the longest phase. Following the mating at the end of the proestrus gestation takes around 21-23 days with a delivery ranging from 55 minutes to 4 hours based on number of puppies to deliver, mainly an average of 1.5 hours [24].

With aging, estrous cycles in rat becomes irregular and constant estrous state may be observed [27]. Around the middle of the life cycle, length of estrous cycle increases due to the age related decrease in progesterone. After six to eighteen months, persistent estrus is noticed which may result in cycles that lasts ten to fourteen days that are called repetitive pseudo pregnancy. The final part of the reproductive system at old age is called persistent anestrus. In persistent estrus, consistent sexual responsiveness is present due to the estradiol levels sustained at tonic level. In repetitive pseudo pregnancy, corpora lutea is maintained beyond its regular time span and it is associated with high progesterone levels. At the persistent anestrus stage, low progesterone levels and high prolactin levels are observed which hinders the LH production [25].

1.4. URINARY BLADDER

The urinary bladder is responsible for emptying out bodies fluid waste product, urine, through the relaxing and contracting of the smooth muscles. The wall of smooth muscles in the bladder is called detrusor. The internal extension of detrusor is called the internal sphincter and the external extension of detrusor is called external urethral sphincter. These are under autonomic and somatic control respectively [28].

Bladder function can be affected by the aging process and urinary flow rate, bladder capacity and bladder compliance decrease because of that [29]. It has been suggested that with aging, micturition dynamics in vivo and contractile strength in vitro can have different responses to neurotransmitters. The micturition volume and frequency of urinating has been observed and it was found that it highly differs in aged rats when compared to young rats [6].

The urination process is a complex process where the central, autonomic and somatic nervous system has to work together. When the bladder fills up to a certain point, the stretch receptors in the bladder sends signals to the nervous system and voluntary urination takes place. On the other hand, there is another circumstance where the detrusor is highly active and causes involuntary contractions when there is only very little urine and it is referred as overactive bladder (OAB). OAB can occur as a result of several factors such as detrusor hyperreflexia, obstructive prostatic hypertrophy and urinary tract infections and unrelated to the cause, spontaneous bladder muscle contractions are observed. This condition generally requires medical treatment and as the syndrome generally has various underlying causes, available treatments are not deemed sufficient. [28].

There have been several proposals in treatment of overactive bladder syndrome using potassium channel openers to inhibit the spontaneous contraction, but due to their effects on cardiovascular systems, they are not being applied routinely. Similar effects were also observed with calcium channels blockers yet their clinical effect was thought to be inadequate [30].

In the bladder, smooth muscle contraction is regulated mainly by the intracellular calcium concentration. Calmodulin mediated activation of myosin light chain (MLC) is activated with the increase in calcium concentration and then the phosphorylation of MLC causes contractions [31].

1.5. ENERGY METABOLISM OF SMOOTH MUSCLES

Main job of the uterus is to keep the fetus inside the uterine cavity during pregnancy. In order to do so, it consists of special construction of connective elements and smooth muscles. In the uterine, there are smooth muscles that surround the uterine cavity and at the outlet of this cavity which is called cervix uteri, there are mainly connective tissues that encircling the cervical canal. The dominating tissues in the uterine body (corpus uteri) is myometrium [32].

There have been many studies on the effects of hormonal changes, such as oxytocin, gestagens and estrogens along with prostaglandins, in the uterine smooth muscles both during the labor and at the initiation of the labor. But the effects of these hormones are thought to be altered by the local factors, such as metabolites showing that the bioenergetics and metabolism of the myometrium are important. This importance comes from the dualistic function of the uterus when pregnant, changing from a sheltering organ prior to labor then changing to a rhythmically contracting tissue to expel the fetus [33]. There are two vital elements for this contraction, presence of an sufficient energy source and increase in the concentration of the intracellular calcium ions. During the contraction, it has been determined that the ATP production from glycolysis rise around 2-3 times and the ATP utilization during this time can increase 3-4 times. It is crucial that ATP is always present because if the energy obtained from the ATP hydrolysis lessens, contractile activity may be compromised [11].

Energy status of the cell is responsible for most of the reaction in the metabolism. Energy charge (EC) is an index that is proportional to the sum of mole fraction of ATP and half the mole fraction of ADP. High EC is responsible for the stimulation of the ATP-utilizing pathways [32]. During this process glucose is the main metabolite [11,33]. Many studies shows that there is a very low energy charge of the uterine muscles. Compared to striated muscles of the rectus, it has been found that there is a high concentration of free adenosines in the uterine muscles which shows that there is a high energy economy [33]. Regardless of the functional state of the uterus low ATP and EC values are higher in uterine muscles compared to striated skeletal muscles as well [34].

1.6. ORGAN BATH

Isolated tissue baths are a classic apparatus that helps in determining the concentration-response relations in many tissues. It has been around over 150 years and due to its versatility and simplicity, it has been an important device for the scientists. Main goal with this device is to measuring the effects of antagonists or inhibitors and their efficacy on the concentration dependent changes in the isometric contractions. Through the advancement of technologies, the analysis and recording of these data have improved and can although the main principle is the same, technology offers more in this area. Over the years, this system has helped with coming up with many medicines to treat diseases such as hypertension, diabetes, bladder dysfunction and many more. They have many advantages in keeping the tissue function while working on the pharmacological changes and allows to understand how they would work in the body [35].

There are several methods used to study the function of myometrium such as cell culture systems and organ baths. Determining whether a substance work at cellular level can be investigated using cell culture system and in organ bath, response of the whole tissue to the reagents can be measured allowing the tissue to function as a tissue [13,35]. Organ bath is generally a heated glass chamber that can hold 5-50 mL of physiological saline solution and aeration with oxygen is required for the strips of tissues in the chamber. In the chamber, dissected strip of tissue is connected to the force transducers which measures tension. Using the *ex vivo* technique contraction performance such as contraction force, frequency and duration can be measured to produce and total work done index and the direct effect of various agents to these parameters. Using isolated tissue strips, physiological reaction of the whole tissue can be measured and thus, the organ bath technique can provide a bridge between the cell culture and *in vivo* work. This results in measuring the effects of various agents in terms of excitatory and inhibitory potential in drug discovery field and their potential in vivo can be evaluated more closely [13].

Although the drug development is intended mainly for humans, its research are generally performed in animal tissues. But measurements of ex-vivo muscles, allows to answer some questions regarding their physiology and pathology and can help understand the responses of certain agents. These agents can vary from known chemical compounds to herbal preparations. Meaning organ bath technique can be used to test any chemical or compound

to be researched and it has been utilized by many researchers to understand its physiological effects[13].

In organ bath, there are also some limitations in the experiments. Main challenge is the dissection and keeping the tissue viability to ensure proper results. It is critical to prevent possible damage when the dissected tissue is being handled, such as during dissection or when hanging to the device [13,35]. In the dissection step, a small strip from the muscle is cut, and it is important to orient the muscle in the correct position and take the strips accordingly. This step is considered difficult for people that are not completely familiar with the tissues or the equipment [13]. There are also some advantages to this technique as well. Since the experiment works is real time, scientists can come to fast conclusion and be able to plan upcoming moves and can easily troubleshoot during the experiment. Also with this techniques, since the experiment is being performed in a bath system, smaller volumes of an drug can be used when compared to in-vivo experiments [35].

2. MATERIALS AND METHODS

Female virgin Sprague-Dawley Rats that are 10, 20, 30 and 40 weeks old (n=3) were obtained from Yeditepe University Medical School Experimental Research Center (YUDETAM). All experiments were approved by the local ethics committee and the animals were kept at a 12:12 h lightness-darkness system.

Experiments were performed in jacketed 20 ml organ cuvettes filled with Krebs solution and aired with 95 percent oxygen and 5 percent carbon dioxide at 37°C. Krebs solution contained NaCl (6.88 g/L), KCl (0.35 g/L), KH_2PO_4 (0.136 g/L), $MgSO_4$ (0.144 g/L), $NaHCO_3$ (1.26 g/L), $MgSO_4$ (0.144 g/L), $CaCl_2$ (0.294 g/L) and glucose (1.98 g/L).

In order to obtain the myometrium and detrusor tissue strips, animals were euthanized with carbon dioxide. Afterwards, making an incision on the abdomen, bladder and uterus were removed and placed in saline solution and then Krebs solution. Following the extraction, the tissues were placed in Petri dish filled with paraffin. There, tissues were dissected and 1cm long strips were obtained. The strips were tied with 4/0 surgical silk suture from the top and the bottom. They were then placed in the tissue bath (BIOPAC MP 35) filled with Krebs solution without $CaCl_2$ and hung to the force displacement transducer hook at the top and stabilized at the bottom hook using the sutures.

At the start of the experiment, tissue bath was filled with Krebs solution without $CaCl_2$, and the program was initiated with 1500 g tension. Tissues were washed 2 times with 20 minute intervals with the $CaCl_2$-free Krebs solution and each time tension was set to 1500 g. Afterwards, the solution was replaced with Krebs with $CaCl_2$ and the tissues were washed and tension was set as performed previously.

In order to begin chemical stimulation, oxytocin and KCl solution were prepared for myometrium and detrusor strips respectively. The oxytocin solution was prepared as dilutions based on 800 mU/L oxytocin supply at the organ bath [36]. The oxytocin solutions ranged from 10^{-3} to 10^{2} 800 mU/L and they were applied to the cuvette with myometrium strips cumulatively with 10 minutes intervals. For the detrusor stimulation 20 to 80 mM KCl concentration was aimed at the tissue bath to see its effect [29,37]. KCl solution was prepared as a 1600 mM stock solution and 50 µl of the stock solution was added cumulatively every 10 minutes.

After application of cumulative doses, the dose where the most effect obtained was observed. The tissues were then washed with the Krebs solution and tension was set to 1500 g once more and waited to come to equilibrium for 10 minutes. Then the specific concentration of the dose was applied and data was collected.

3. RESULTS

Results for the subjects below was obtained from Biopac program, their numbering is explained in the Table 3.1. for clarity. The related graphs for both myometrium and detrusor are shown in the Figure 3.1.-3.30

Table 3.1. Rat ages and subject numbering

Rat ages	10 weeks old	20 weeks old	30 weeks old	40 weeks old
Subject numbers	Subject 10.1	Subject 20.1	Subject 30.1	Subject 40.1
	Subject 10.2	Subject 20.2	Subject 30.2	Subject 40.2
	Subject 10.3	Subject 20.3	Subject 30.3	Subject 40.3

For Subjects 10.1, 30.1, 30.2 and 20.1, different method was applied for both myometrium and detrusor muscles and for detrusor only in Subject 20.2. After not being able to obtain proper results, a new method was determined after these experiments and they will not be included in the study. All experimental data graphs are shown in section 3.1. and 3.2 for myometrium and detrusor tissue respectively.

In Subject 10.2, regular contractions were observed in both Krebs solutions and chemical stimulation in myometrium but in subject 10.3, there were no contractions in the desired way showing that the tissue might have lost its viability.

All myometrium tissues showed a good contraction rhythm in Subjects of week 20. Good contractions were also observed in Subject 30.3. In subjects of week 40, there were no significant difference in the final two doses of the oxytocin, thus a smaller concentration was given compared to other subjects.

For detrusor tissue, a different contraction pattern is observed and after application of higher doses, it can be seen that the tissue is not able to relax, but hold its tension at the contraction point. In subject 10.3, detrusor tissue has lost it viability after a while, and no extra contractions were observed after this point. In 20.3 and 30.3 a higher contraction was observed in the cumulative method rather than the application of one single dose. Strips in

Subjects 40.2 and 40.3 showed good contractions with the same dose, but in Subject 40.1, full data was not obtained due to tissue losing viability.

In order to compare the work done by the muscles during different steps of the experiment, work values were calculated in MATLAB. This was done by the initial calculation of displacement versus time graph, where displacement values were assumed by taking the length of the uterus muscle as 9 cm and assuming the expelling of the fetus is done in 500 seconds. Afterwards, using the force versus time graphs that were obtained during the experiment, area under the force versus displacement graph was calculated to find the work done by the muscles and are listed in Table 3.2. and Table 3.3. below.

Table 3.2. Work values (J) during myometrium contractions throughout the experiment

Subjects		**10.2**	**10.3**	**20.2**	**20.3**	**30.3**	**40.1**	**40.2**	**40.3**
Experiment steps	**Krebs solution w/o $CaCl_2$**	0.045J	0.087	0.078	0.088	0.075	0.063	0.047	0.067
	Krebs Solution with $CaCl_2$	0.052	0.131	0.110	0.123	0.128	0.147	0.121	0.102
	10^{-3} mU/L Oxytocin	0.078	0.099	0.144	0.126	0.114	0.158	0.150	0.149
	10^{-2} mU/L Oxytocin	0.073	0.111	0.184	0.123	0.115	0.175	0.164	0.207
	10^{-1} mU/L Oxytocin	0.075	0.140	0.208	0.144	0.179	0.180	0.187	0.261
	10 mU/L Oxytocin	0.094	0.160	0.230	0.111	0.189	0.167	0.200	0.246
	10^{2} mU/L Oxytocin	-	-	0.195	0.080	--	-	-	-
	Reapplication of 10^{-1} mU/L Oxytocin	0.093	-	0. 170	0.130	-	0.166	0.165	0.204
	Reapplication of 10 mU/L Oxytocin	-	0.162	-	0.131	0.164	-	-	-

Table 3.3. Work values (J) during detrusor contractions throughout the experiment

Subjects		10.2	10.3	20.3	30.3	40.1	40.2	40.3
Experiment steps	**Krebs solution without $CaCl_2$**	0.101	0.061	0.068	0.157	0.132	0.059	0.079
	$CaCl_2$ krebs	0.105	0.110	0.089	0.095	0.153	0.097	0.161
	4 µL KCl	0.123	0.102	0.090	0.108	0.138	0.122	0.197
	8 µL KCl	0.117	0.107	0.120	0.128	0.169	0.167	0.252
	12 µL KCl	0.112	0.119	0.154	0.151	0.231	0.207	0.267
	16 µL KCl	0.118	0.128	0.187	0.178	0.254	0.211	0.317
	20 µL KCl	0.125	0.139	0.251	0.204	0.310	0.256	0.379
	24 µL KCl	0.107	0.164	0.299	0.245	0.293	0.281	0.391
	28 µL KCl	0.121	0.199	0.316	0.266	0.125	0.278	0.375
	32 µL KCl	0.090	0.217	0.320	0.269	0.133	0.285	0.386
	36 µL KCl	-	0.217	0.333	0.224	0.070	0.306	0.403
	40 µL KCl	-	0.224	0.356	0.219	0.139	0.325	0.416
	44 µL KCl	-	0.245	0.362	0.225	0.154	0.336	0.417
	48 µL KCl	-	0.275	0.369	0.230	0.162	0.343	0.420
	52 µL KCl	-	0.298	0.374	0.235	0. 169	0.359	0.416
	56 µL KCl	-	0.313	0.372	0.237	0.178	0.360	0.412
	60 µL KCl	-	0.327	-	-	-	-	-
	64 µL KCl	-	0.333	-	-	-	-	-
	68 µL KCl	-	0.337	-	-	-	-	-
	72 µL KCl	-	0.344	-	-	-	-	-
	Reapplication of 52 µL KCl	-		0.244	-	0.160	0.250	0.286
	Reapplication of 56 µL KCl	-	0.278		-	-		

3.1. MYOMETRIUM CONTRACTION GRAPHS

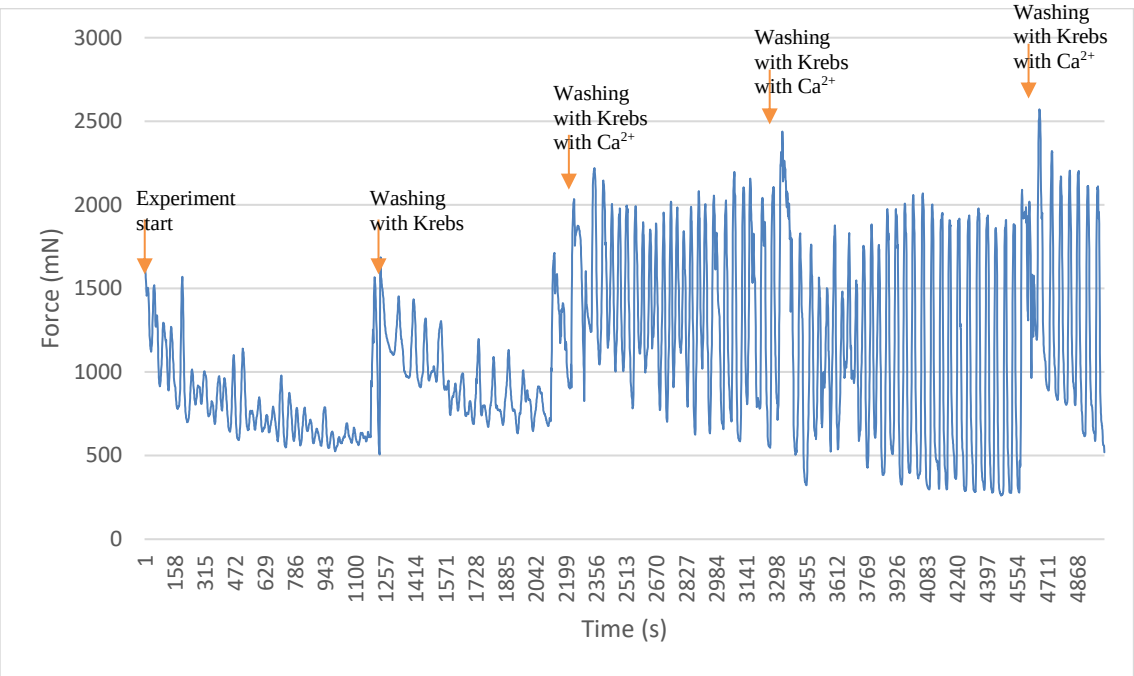

Figure 3.1. Contractions in Krebs solution with and without Ca2+ of Subject 10.2

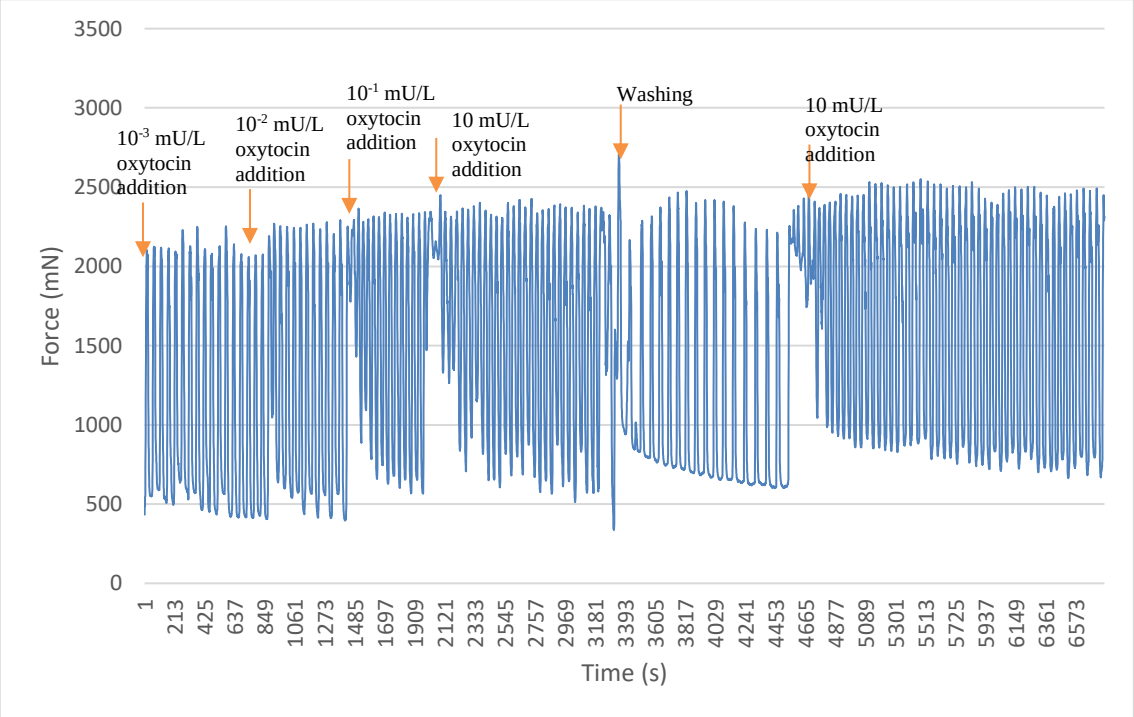

Figure 3.2. Chemically induced myometrium contractions of Subject 10.2

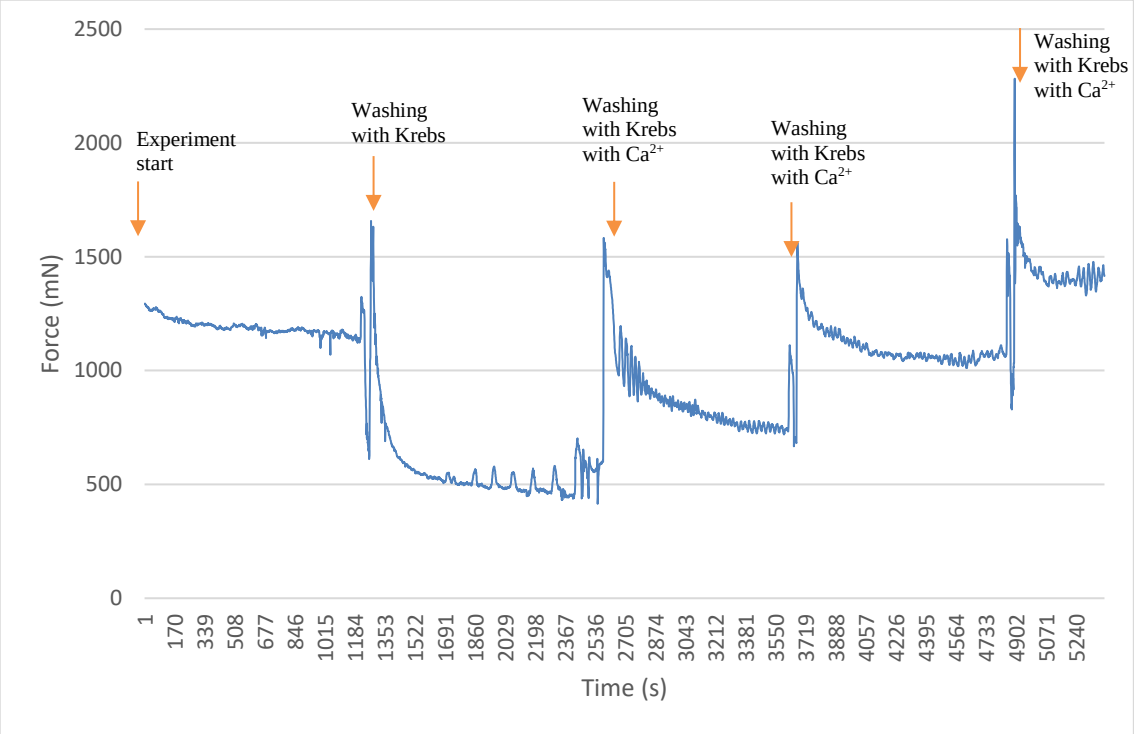

Figure 3.3. Myometrium contractions in Krebs solution with and without Ca2+ of Subject 10.3

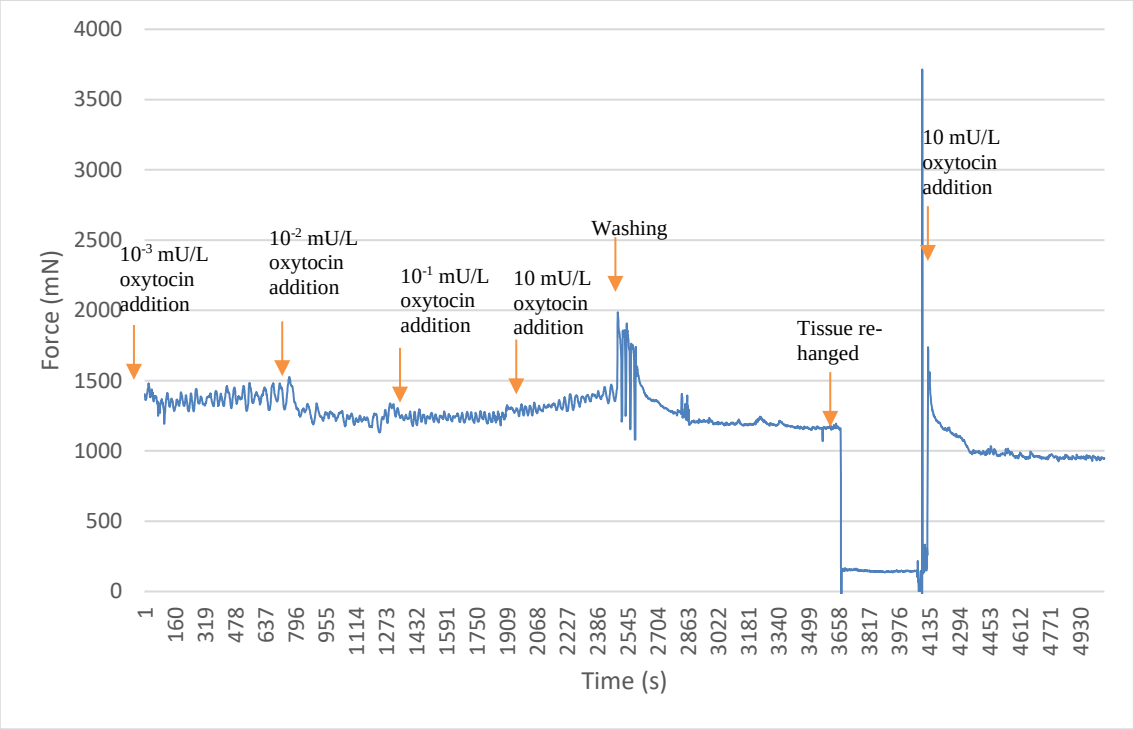

Figure 3.4. Chemically induced myometrium contractions of Subject 10.3

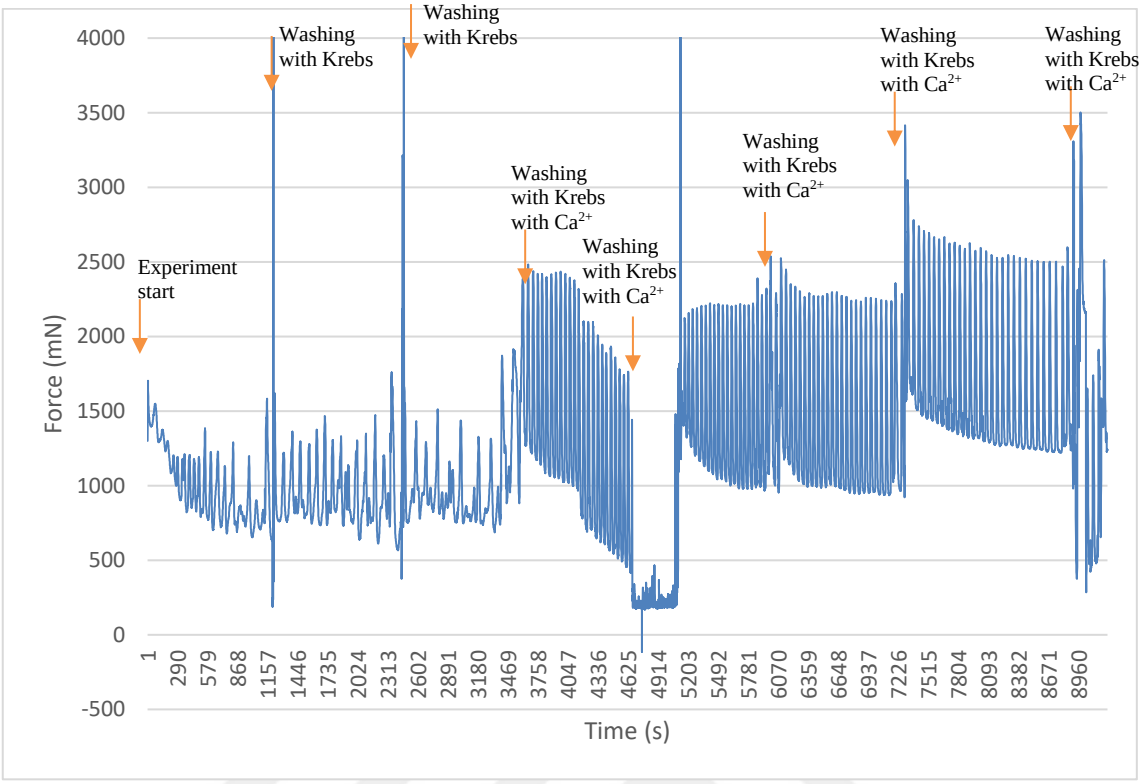

Figure 3.5. Contractions in Krebs solution with and without Ca2+ of Subject 20.2

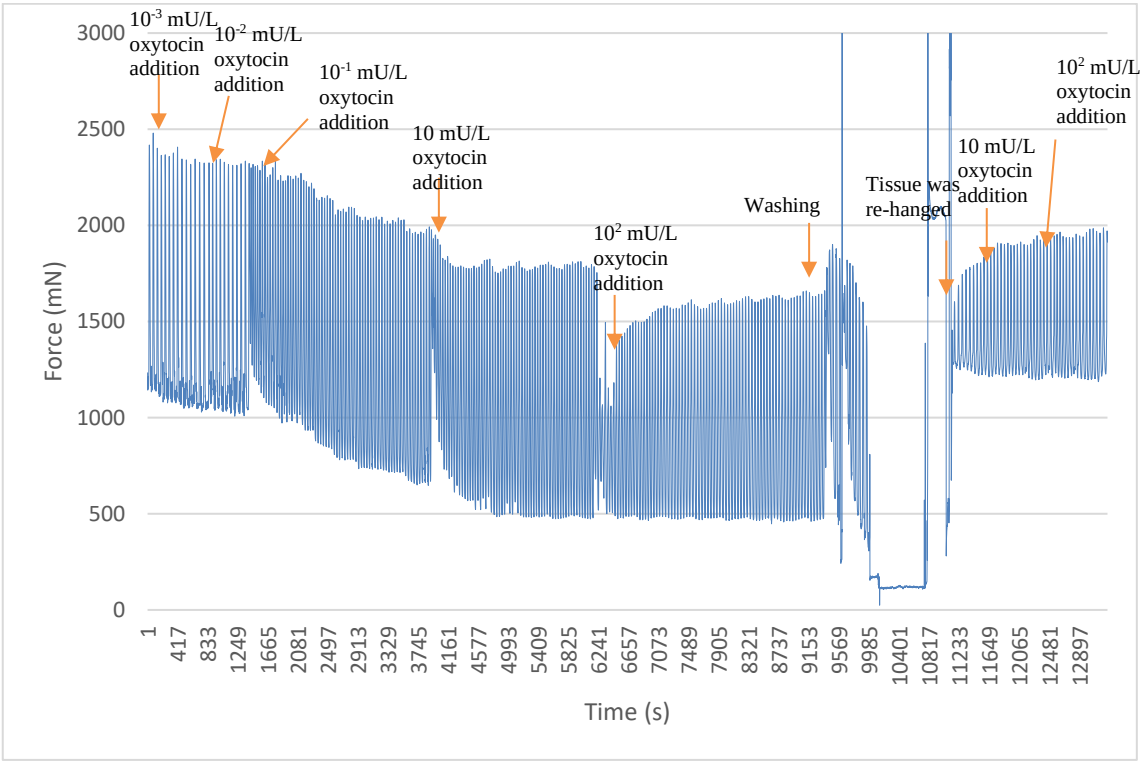

Figure 3.6. Chemically induced myometrium contractions of Subject 20.2

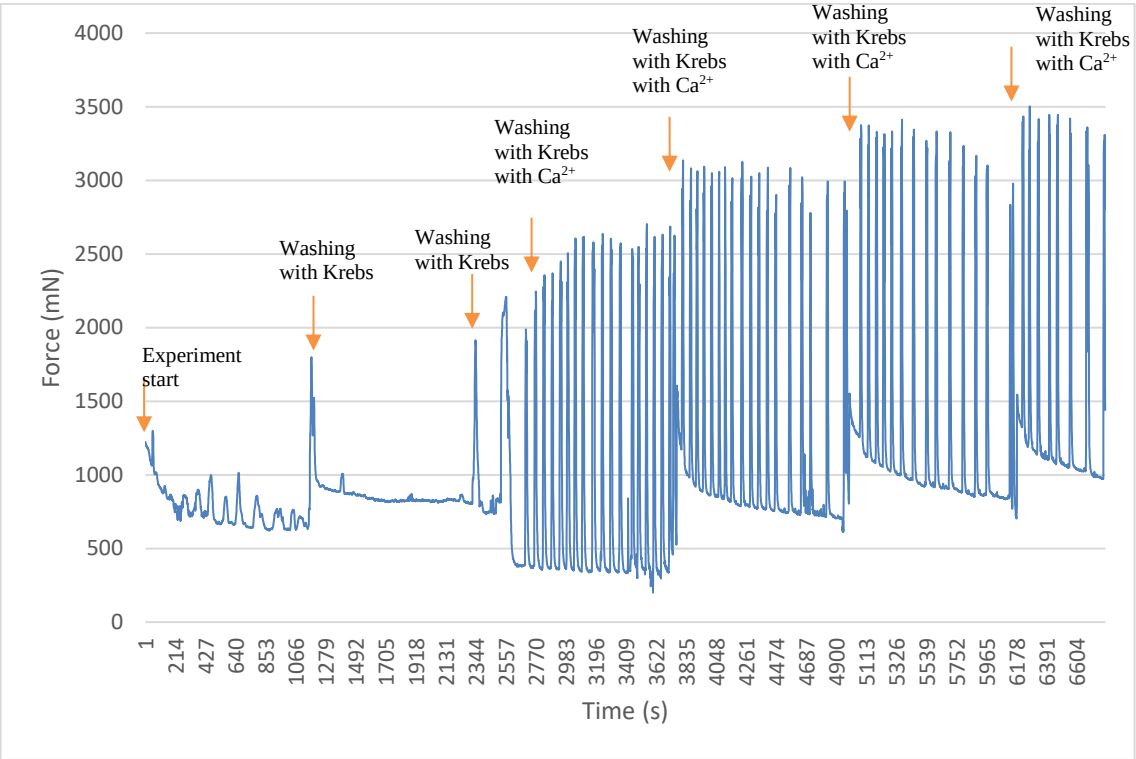

Figure 3.7. Contractions in Krebs solution with and without Ca2+ of Subject 20.3

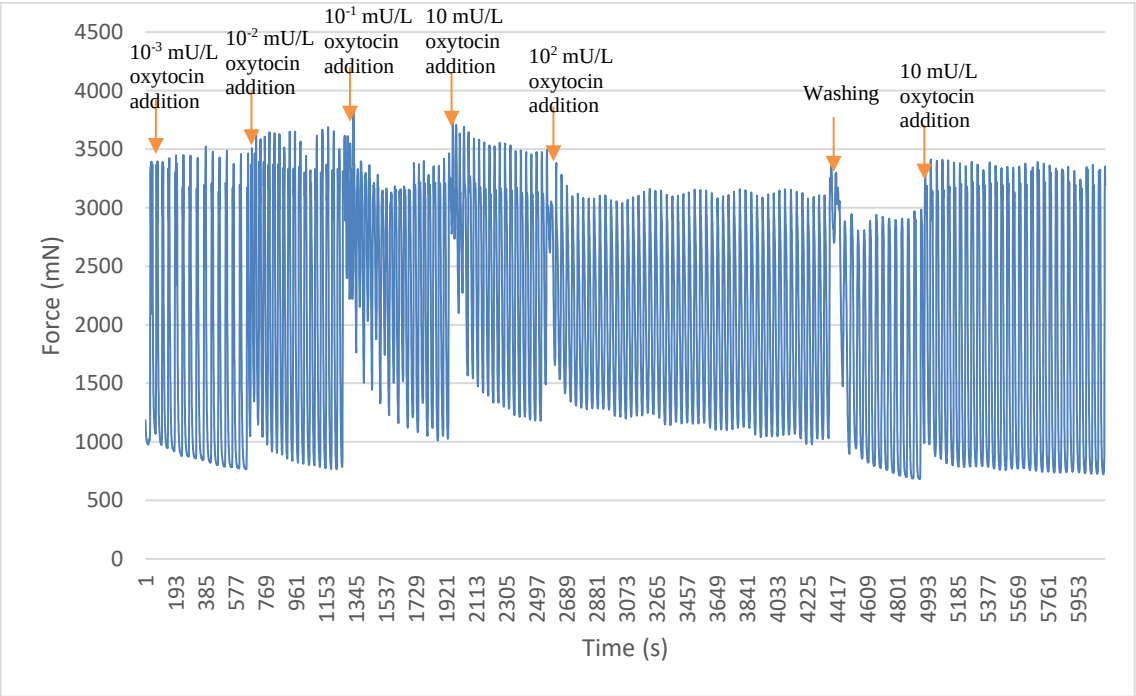

Figure 3.8. Chemically induced myometrium contractions of Subject 20.3

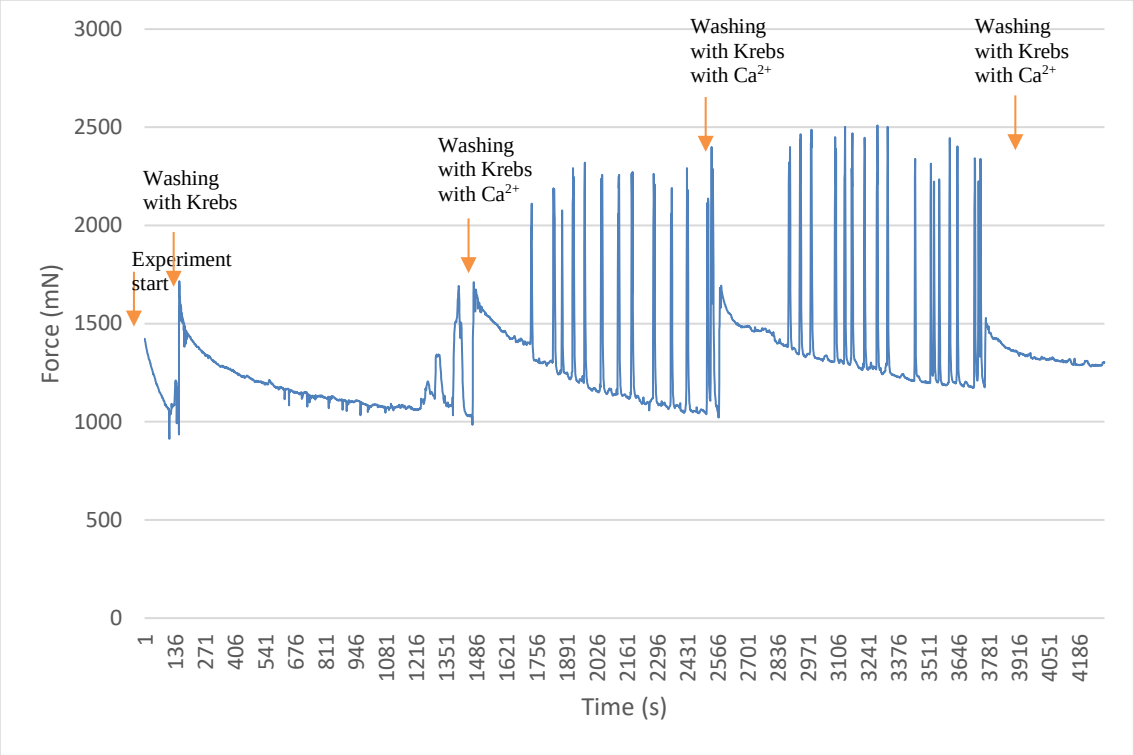

Figure 3.9. Contractions in Krebs solution with and without Ca2+ of Subject 30.3

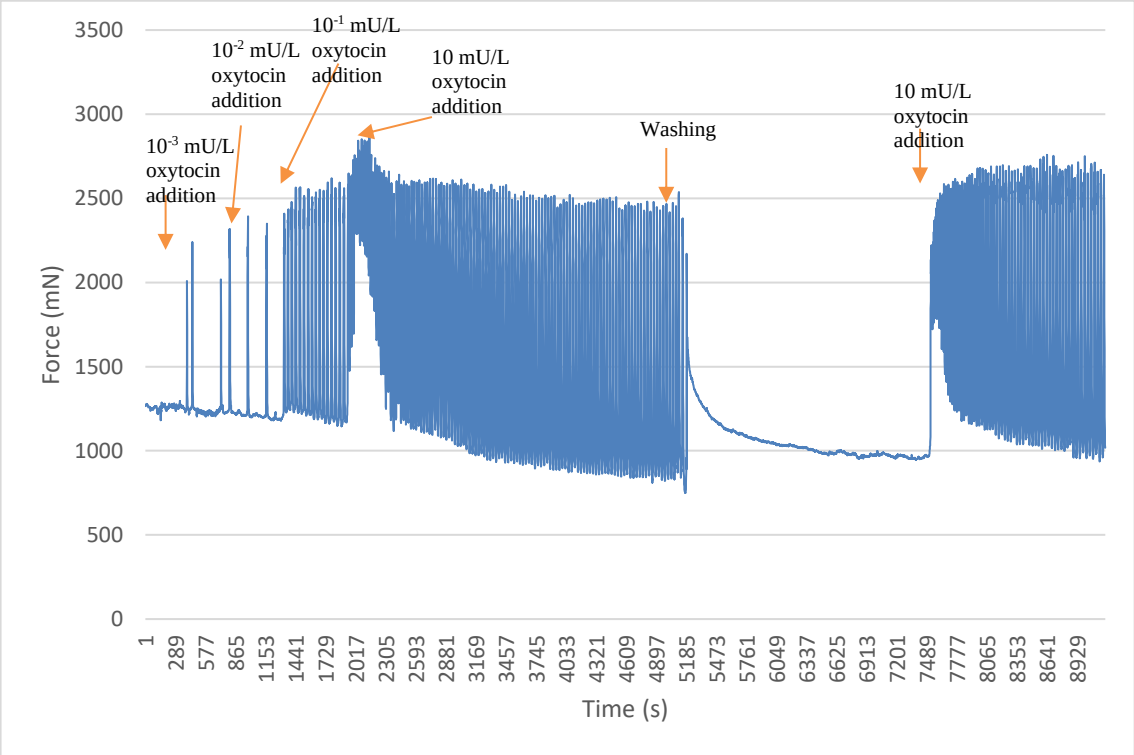

Figure 3.10. Chemically induced myometrium contractions of Subject 30.3

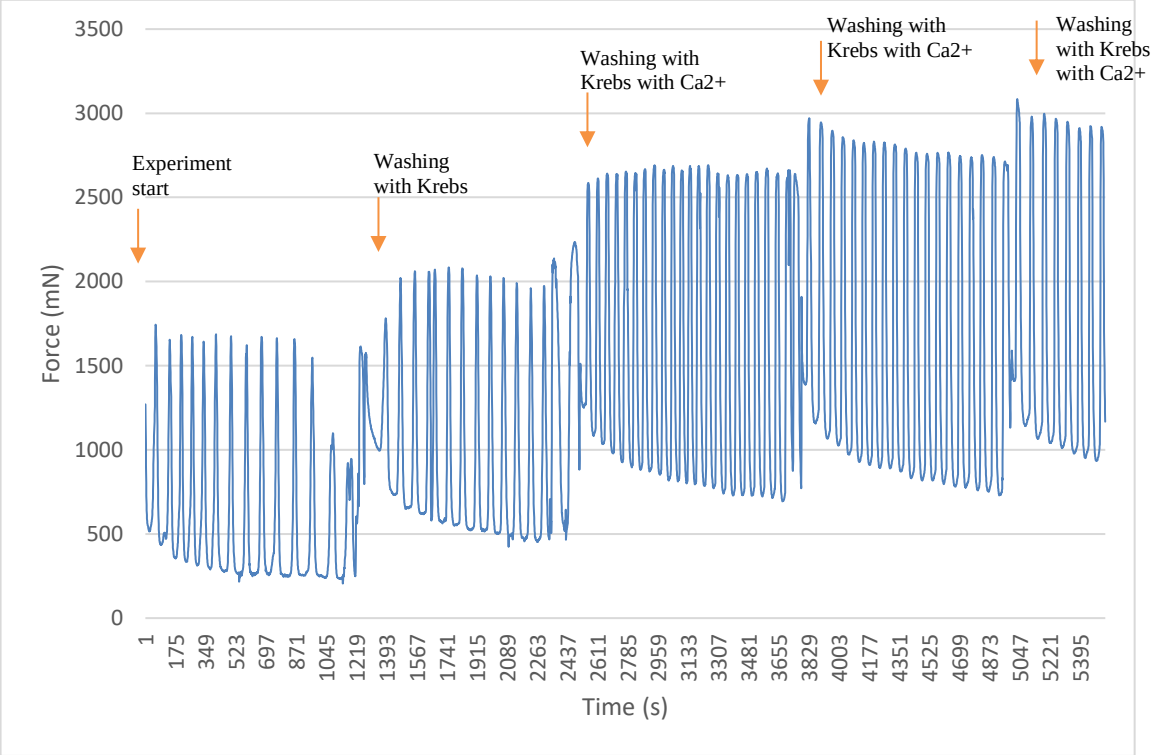

Figure 3.11. Contractions in Krebs solution with and without Ca2+ of Subject 40.1

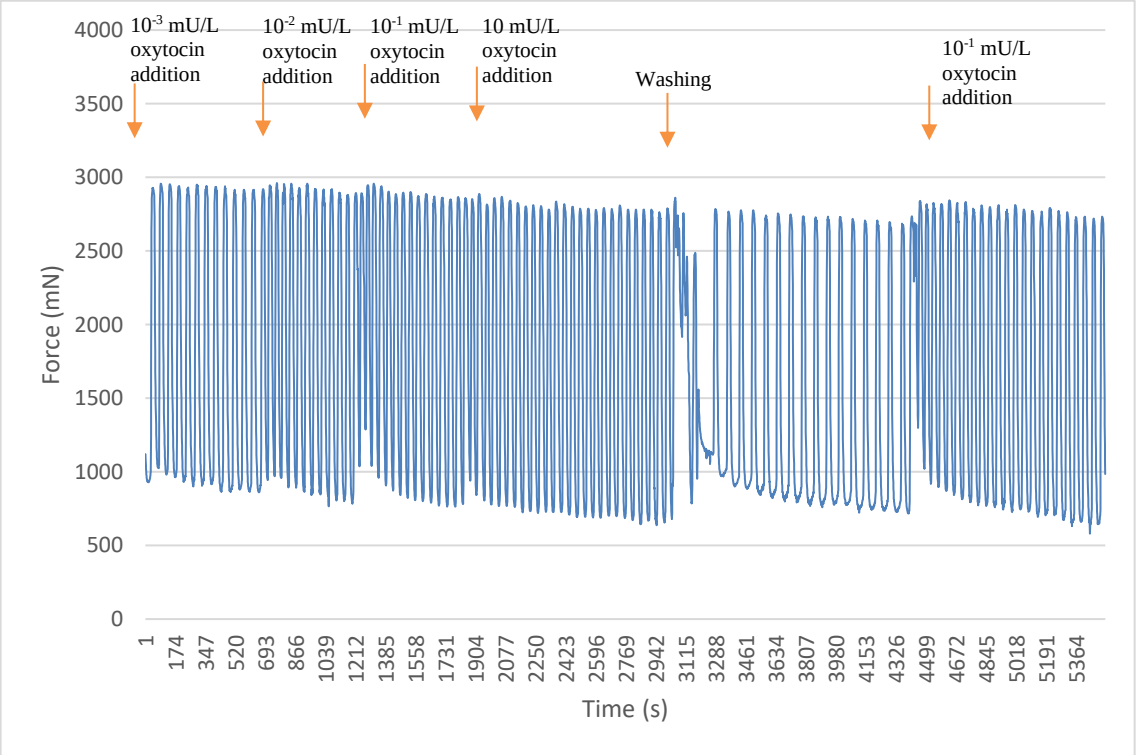

Figure 3.12. Chemically induced myometrium contractions of Subject 40.1

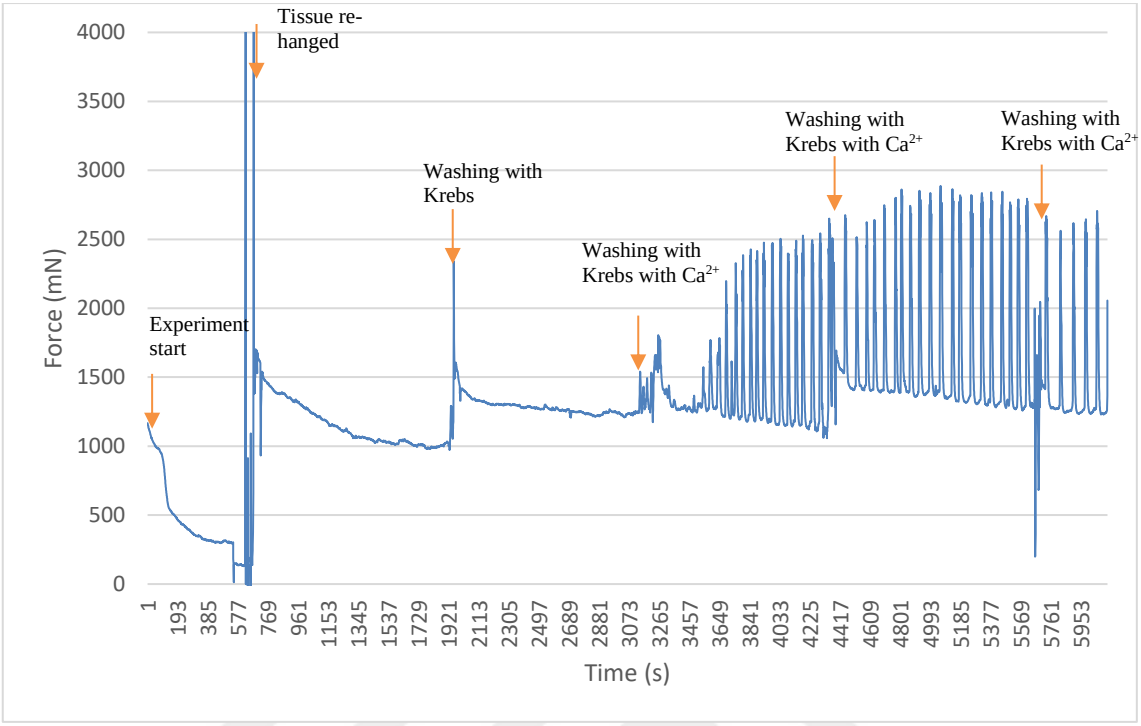

Figure 3.13. Contractions in Krebs solution with and without Ca2+ of Subject 40.2

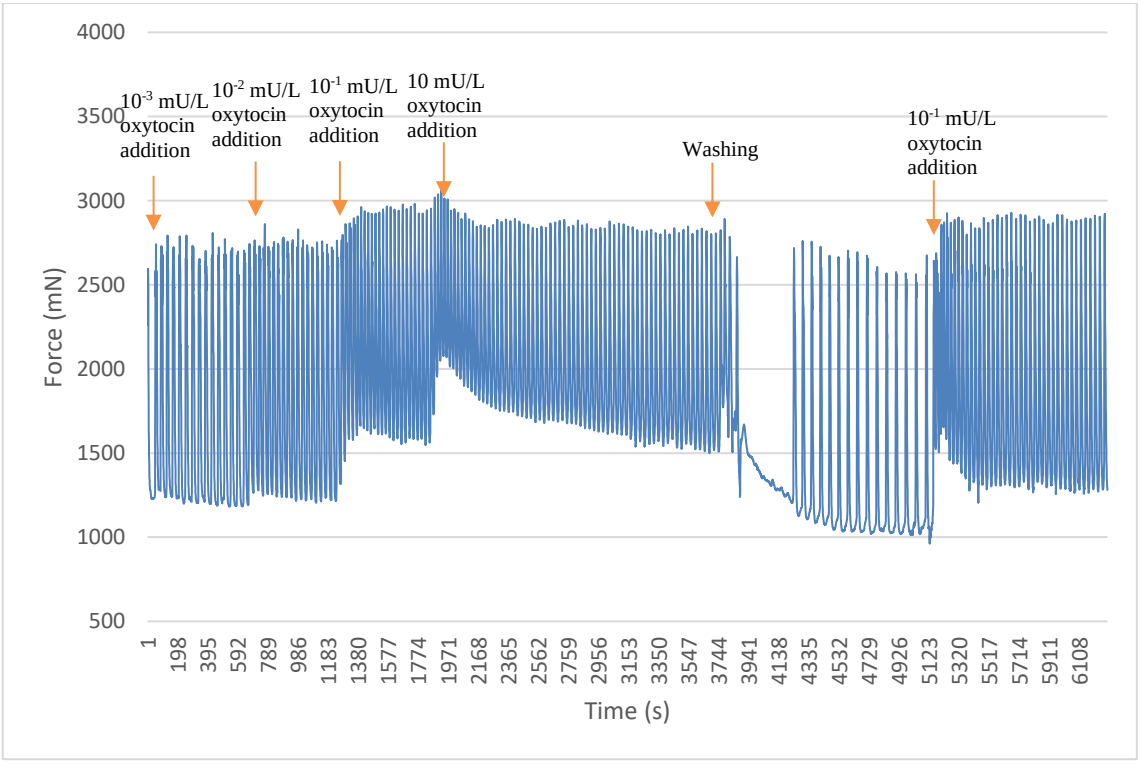

Figure 3.14. Chemically induced myometrium contractions of Subject 40.2

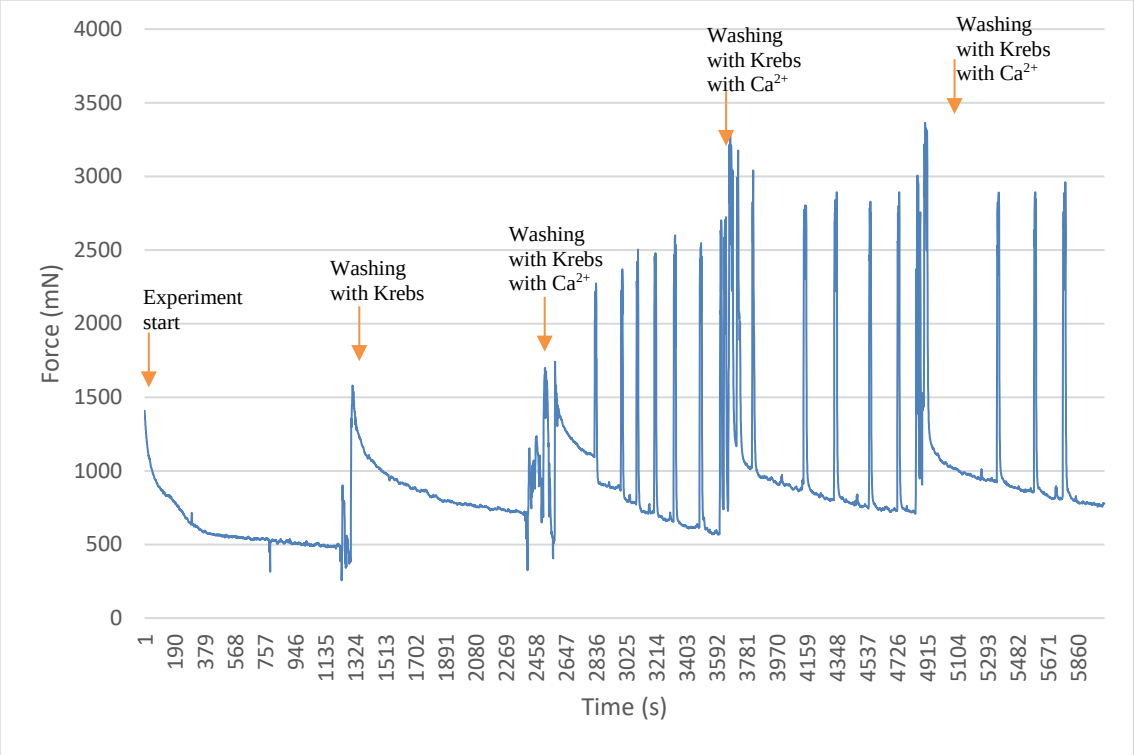

Figure 3.15. Contractions in Krebs solution with and without Ca2+ of Subject 40.3

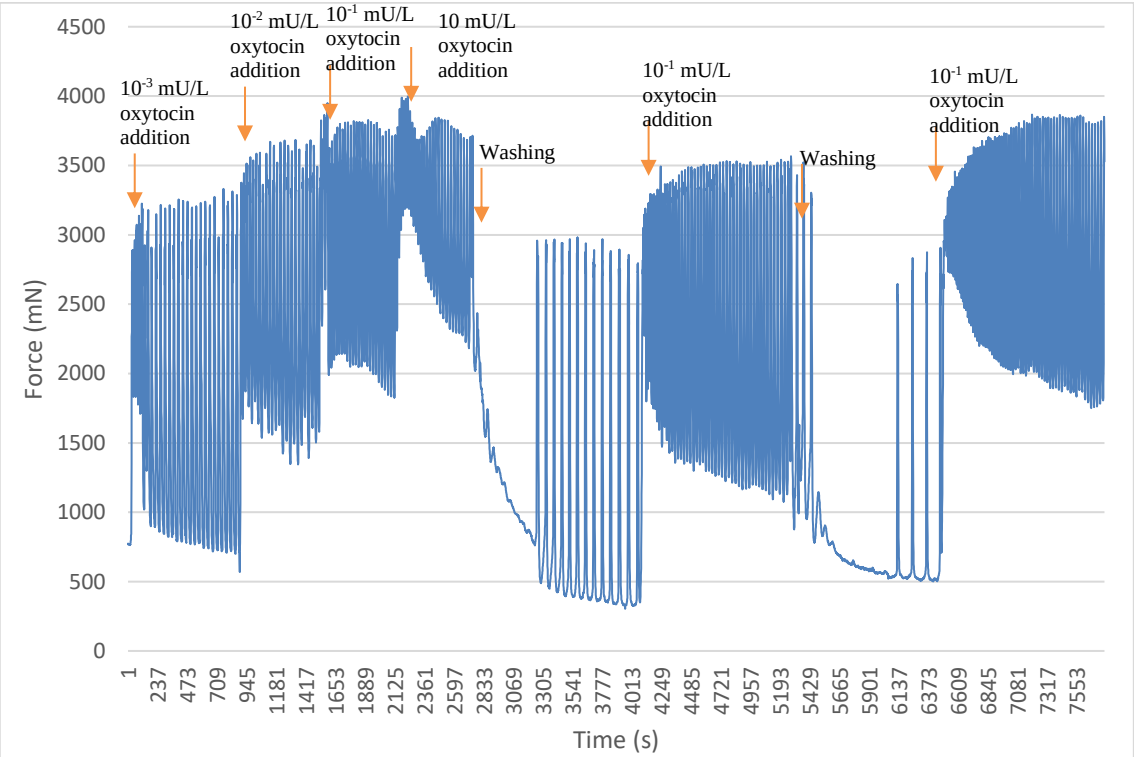

Figure 3.16. Chemically induced myometrium contractions of Subject 40.3

3.2. DETRUSOR CONTRACTION GRAPHS

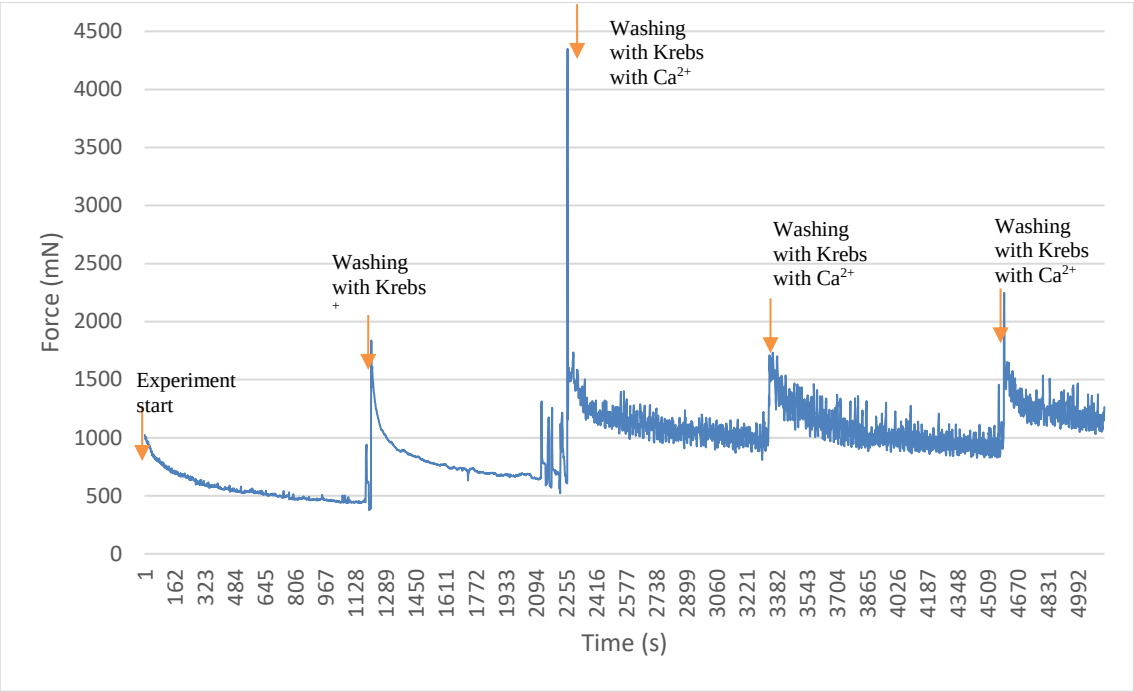

Figure 3.17. Detrusor contractions in Krebs solution with and without Ca^{2+} of Subject 10.2

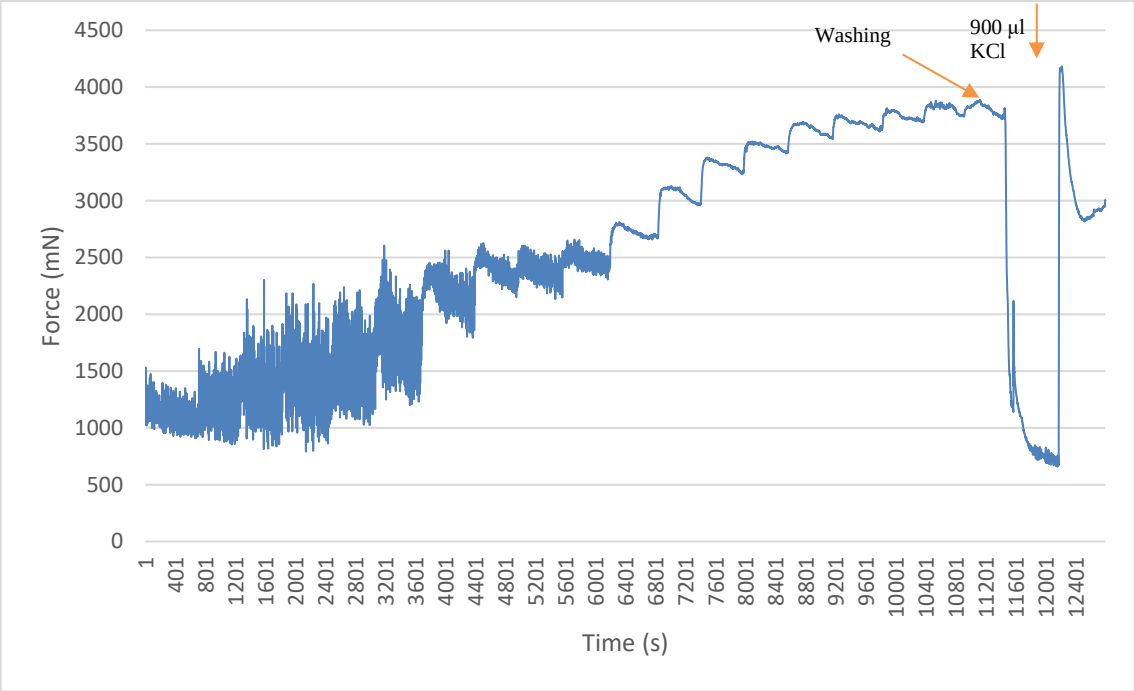

Figure 3.18. Chemically induced detrusor contractions of Subject 10.2

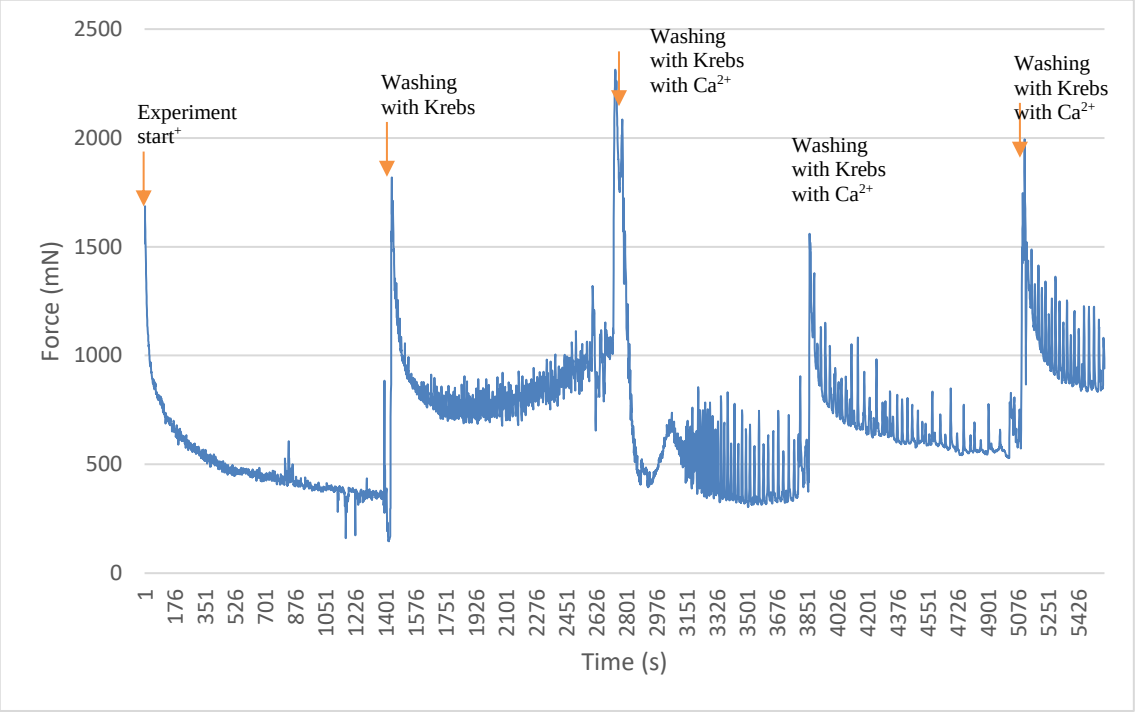

Figure 3.19. Detrusor contractions in Krebs solution with and without Ca^{2+} of Subject 10.3

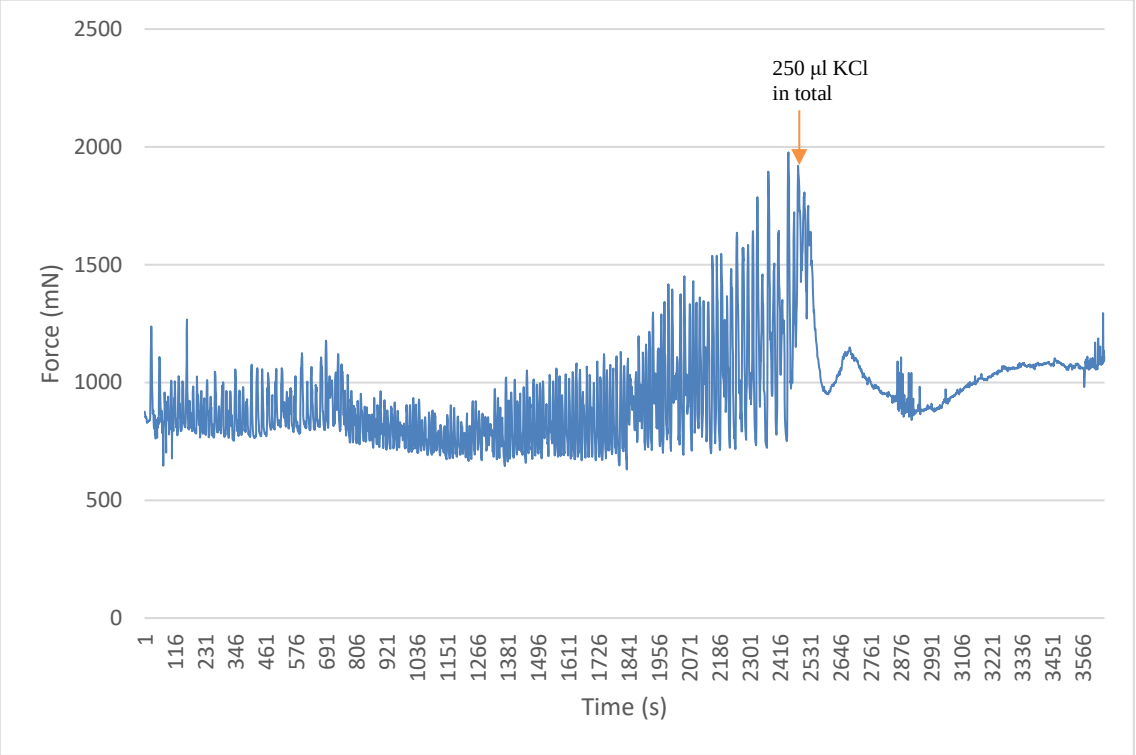

Figure 3.20. Chemically induced detrusor contractions of Subject 10.3

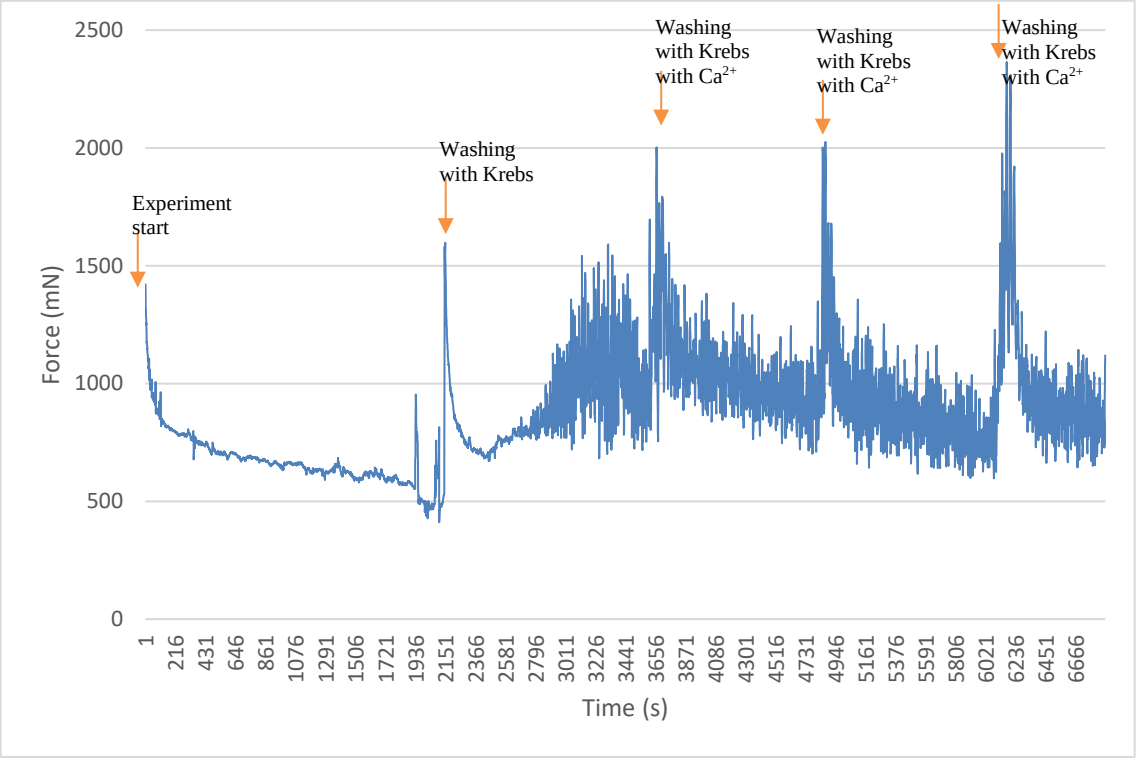

Figure 3.21. Detrusor contractions in Krebs solution with and without Ca^{2+} of Subject 20.3

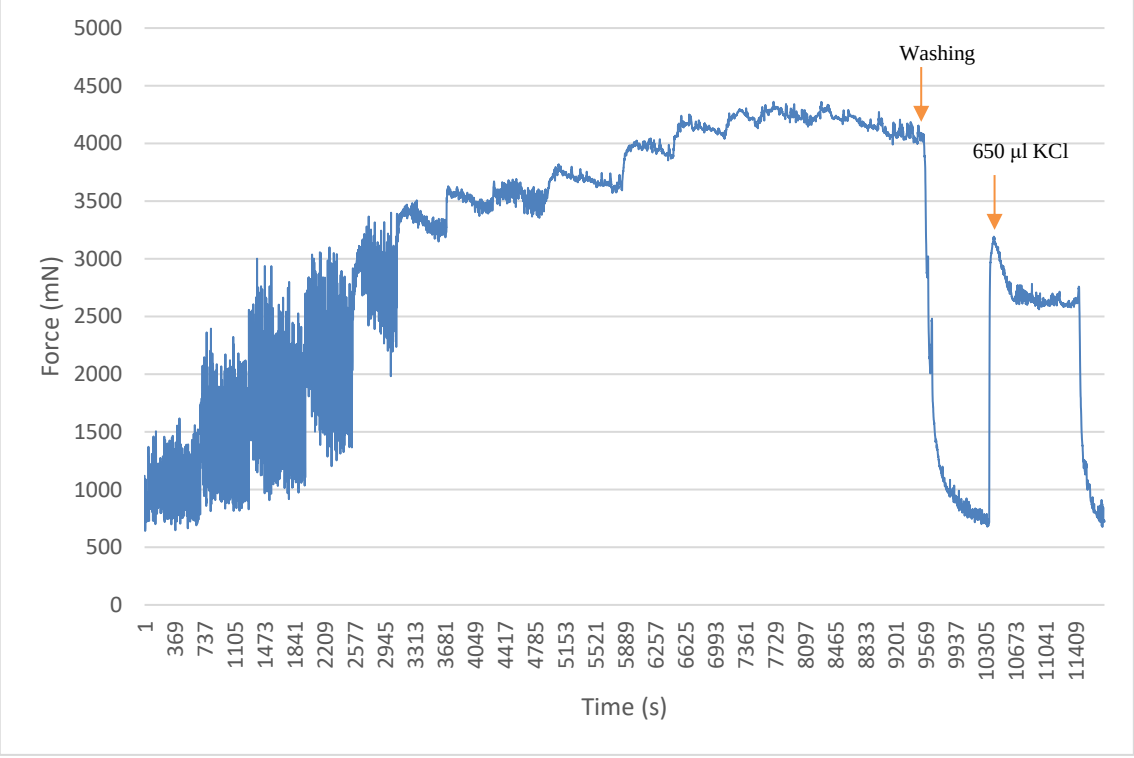

Figure 3.22. Chemically induced detrusor contractions of Subject 20.3

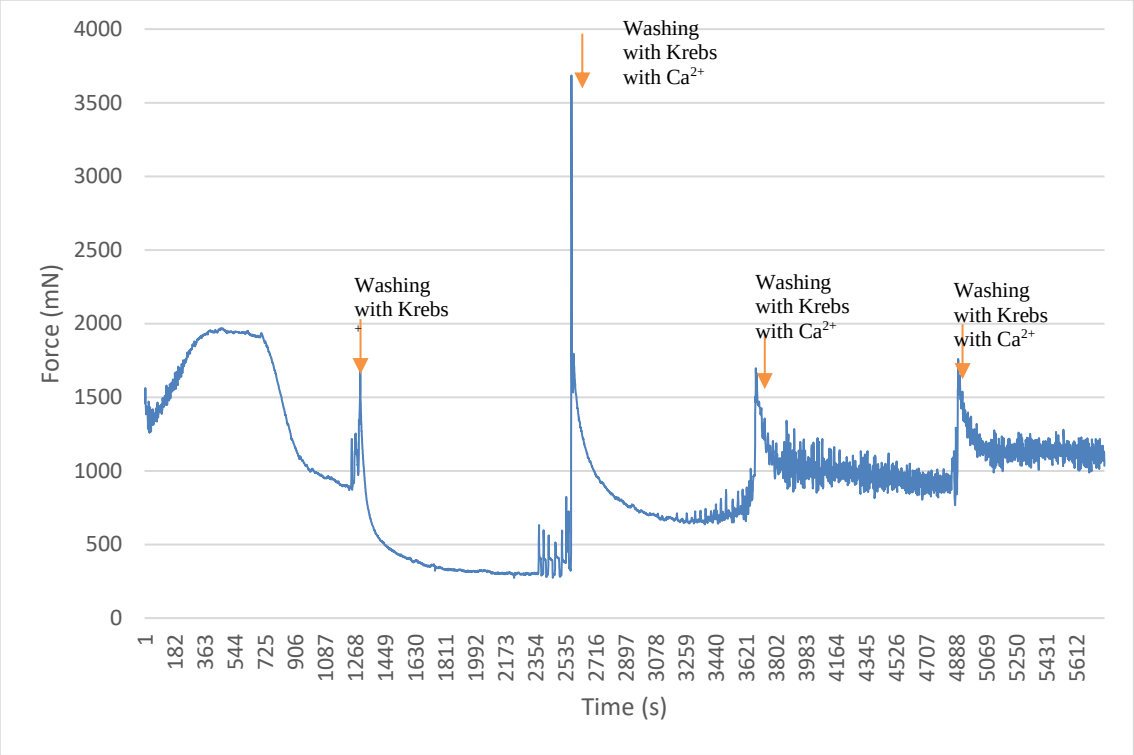

Figure 3.23. Detrusor contractions in Krebs solution with and without Ca^{2+} of Subject 30.3

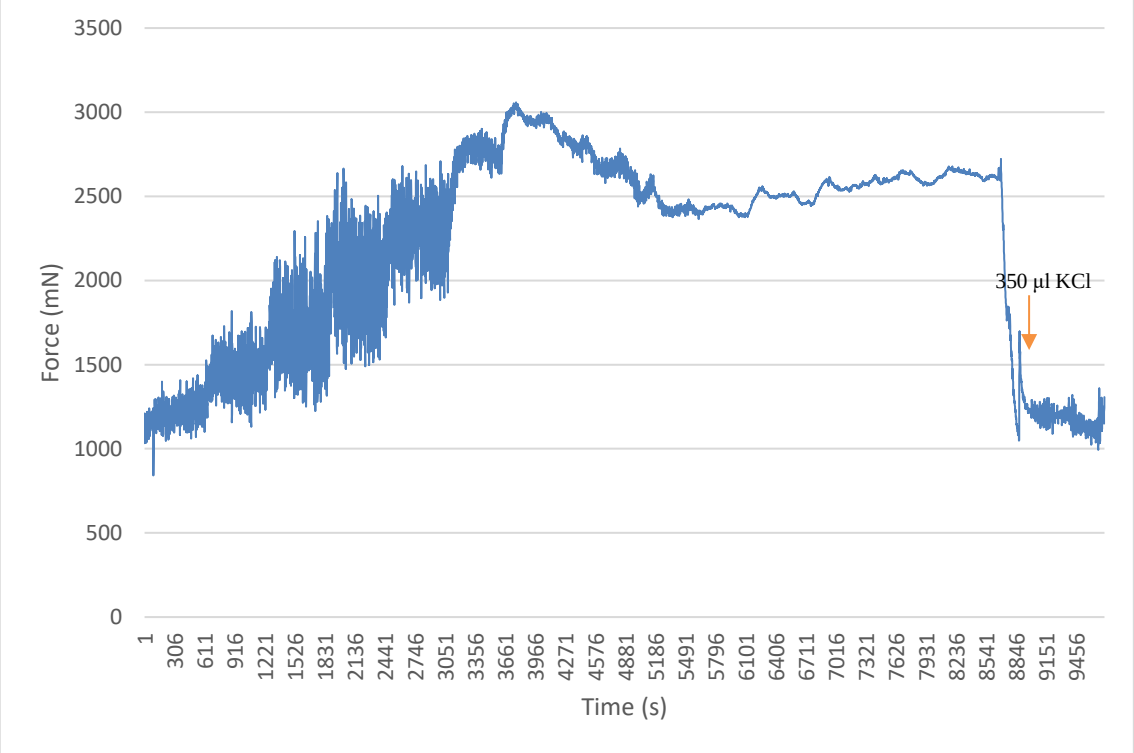

Figure 3.24. Chemically induced detrusor contractions of Subject 30.3

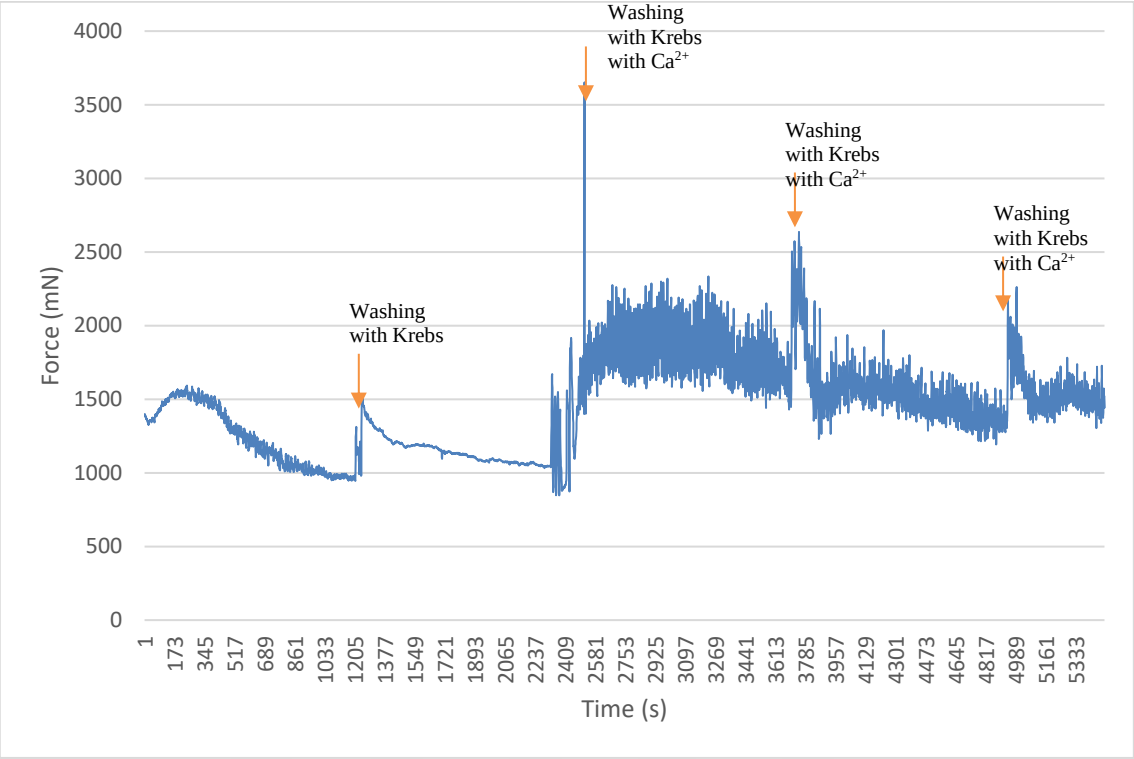

Figure 3.25. Detrusor contractions in Krebs solution with and without Ca^{2+} of Subject 40.1

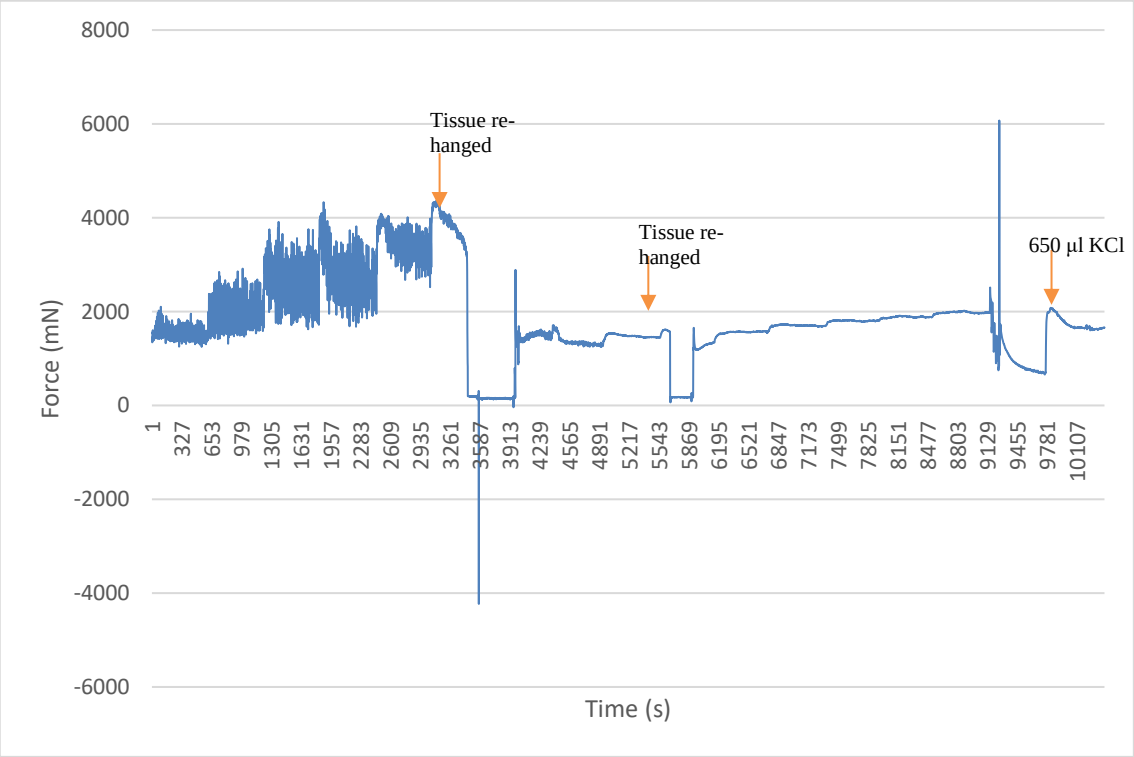

Figure 3.26. Chemically induced detrusor contractions of Subject 40.1

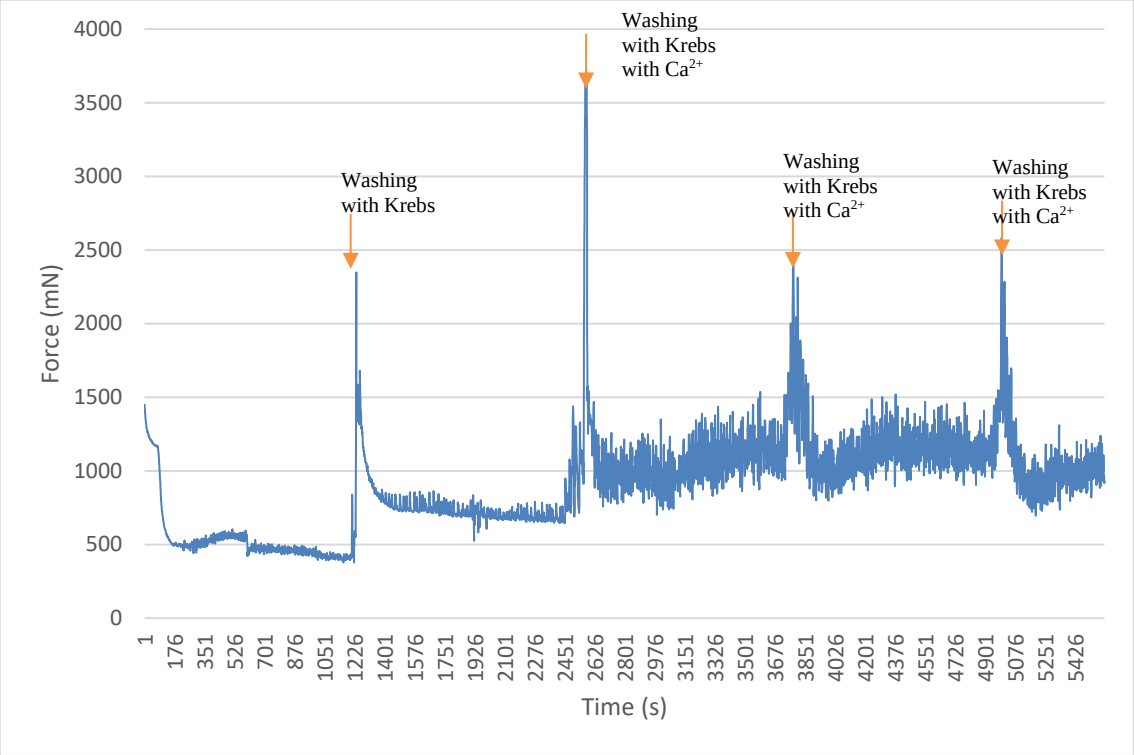

Figure 3.27. Detrusor contractions in Krebs solution with and without Ca^{2+} of Subject 40.2

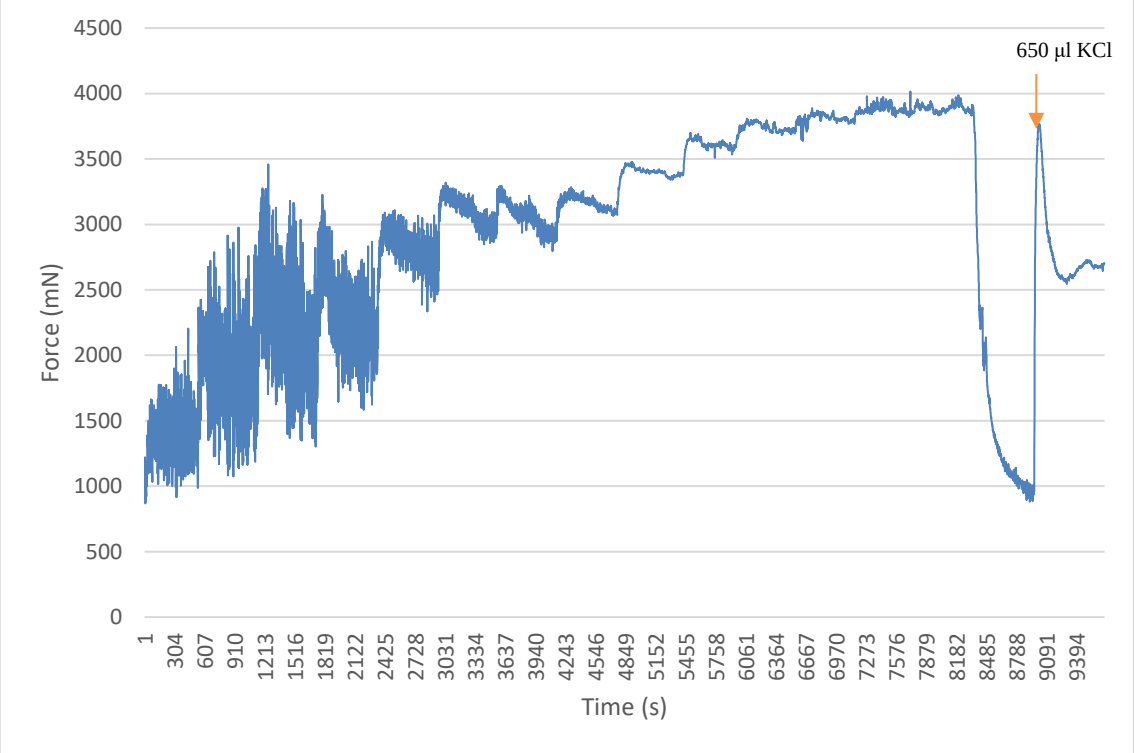

Figure 3.28. Chemically induced detrusor contractions of Subject 40.2

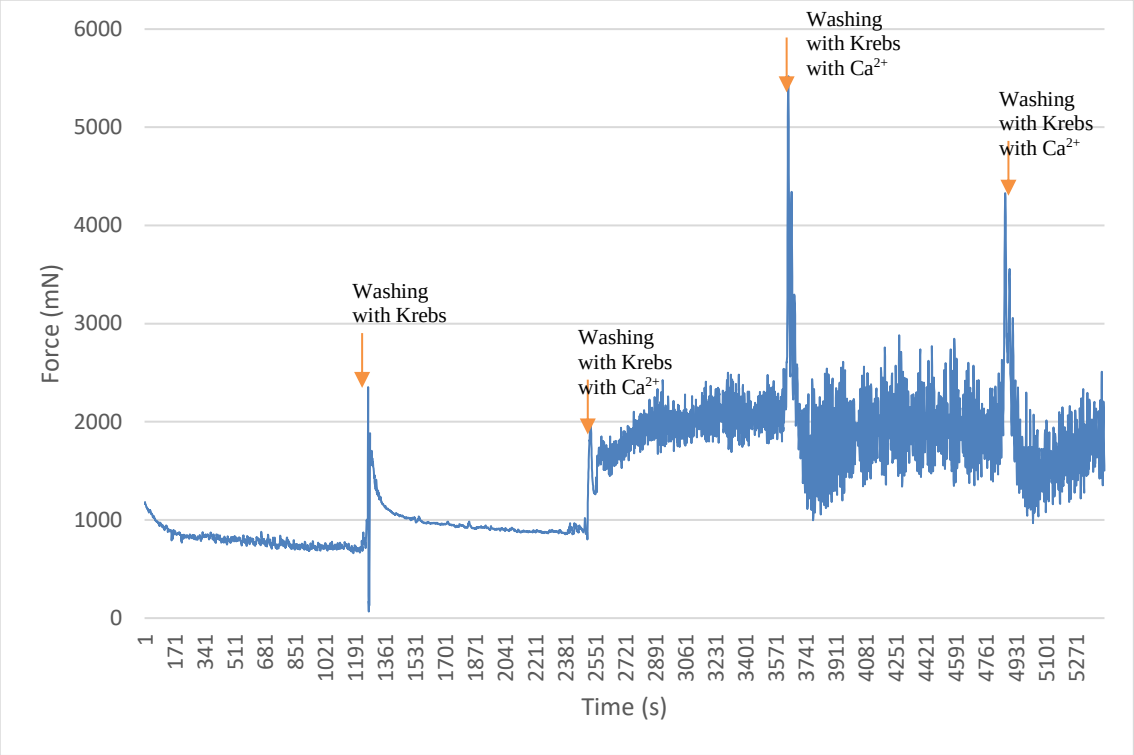

Figure 3.29. Detrusor contractions in Krebs solution with and without Ca^{2+} of Subject 40.3

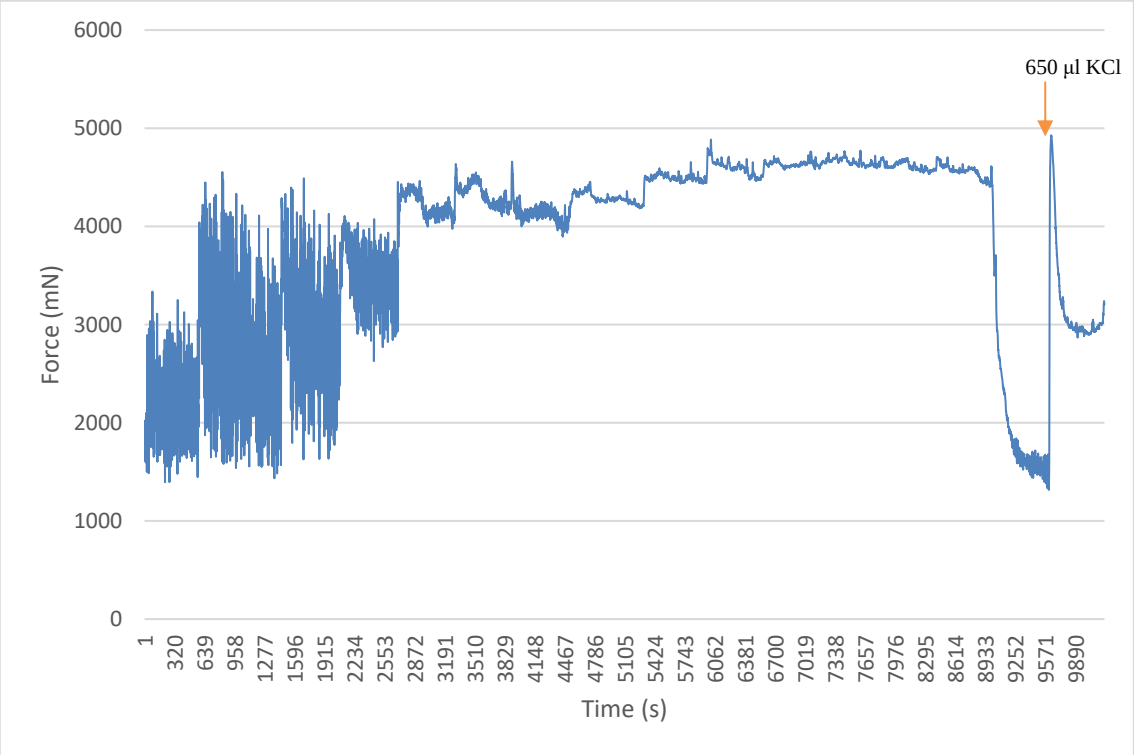

Figure 3.30. Chemically induced detrusor contractions of Subject 40.3

4. DISCUSSION

Organ bath system helps with treating diseases such as hypertension, diabetes, bladder dysfunction and many more by helping to find different medicines. During the experiment the tissue function is kept in the apparatus while working on the pharmacological changes and helps to determine how they would work in the body [35]. Since the experiment works is real time, scientists can come to fast conclusion and be able to plan upcoming moves and can easily troubleshoot during the experiment. Also the experiment is being performed in a bath system, smaller volumes of an drug can be used when compared to in-vivo experiments, which are considered to be the advantages of the technique [35].

Due to the increase in importance to career, higher age when giving birth which has made treating older females that have difficulty when conceiving and giving birth an important part of today's medicine [3]. Reproductivity in females includes individual variability but in general, reproductive senescence is observed in ages 40 to 60 [4]. Also, around 10 percent of the elderly population is considered to have urinary incontinence caused by the abnormal spontaneous contraction of the bladder muscles and treatments for this have not been completely optimized [6]. In this study, it was aimed to observe the changes of the muscle contraction in myometrium and detrusor tissues.

Oxytocin was used in the experiment to observe its effect during contraction of different aged muscles. It is possible that almost one third of women will require augmentation of labor, which is mainly oxytocin [13]. In this experiment it was observed that different amounts can result in different levels of contractility. Especially in the 40-week-old subjects, it was seen that higher oxytocin did not cause extra contractile force compared to younger subjects. Observing the work values of different subject, it was seen that the younger subjects had to perform less work than the older individuals in order to expel a puppy through the same length of the uterus horn. Also, the experiment shows that the higher concentrations of oxytocin, increases the work done by the muscle as a result of chemical stimulation.

KCl was used as an inducer in detrusor muscles as it helps contraction through the potassium channels. In 10 week old subjects, increase in KCl concentration showed higher contractility, but this was not observed in 40 week old's as they did not have the contractility increase in

higher concentrations. Similar to myometrium, younger tissues had a higher work values when compared to older subjects.

5. CONCLUSIONS

Aim of this study was to comprehend the differences between the contraction efficiency of the myometrium and detrusor tissues in various age groups. Rats were used as they are ideal to represent the mechanism in humans. Results shows that increased age has a significant effect on both the contractile mechanism of the muscles and reaction to the chemical stimulants.

In both tissues, it was observed that higher work efficiency is observed in younger subjects. This shows that the process of giving birth as well as the urination process is much more efficient in younger ages. Although application of chemical stimulants may be beneficial for aiding in these processes in humans, it is important to take the age of subjects in to account as age related changes in the human body may limit their usage.

REFERENCES

1. Malik M, Roh M, England SK. Uterine contractions in rodent models and humans. *Acta Physiol*. 2021;231(4):1–17.

2. Dunford JR, Lutton EJ, Atia J, Blanks AM, van den Berg HA. Computational physiology of uterine smooth muscle. *Sci Prog*. 2019;102(2):103–26.

3. Meldrum DR. Female reproductive aging - Ovarian and uterine factors. *Fertil Steril*. 1993;59(1):1–5.

4. Hanna CW, Bretherick KL, Gair JL, Fluker MR, Stephenson MD, Robinson WP. Telomere length and reproductive aging. *Hum Reprod*. 2009;24(5):1206–11.

5. Keefe DL, Liu L. Telomeres and reproductive aging. *Reprod Fertil Dev*. 2009;21(1):10–4.

6. Saiko M, Kondo A, Gotoh M, Kato K. Age Related Changes In The Rat Detrusor Muscle: The Contractile Response To Inorganic Ions. *J Urol*. 1991;146:891–4.

7. Lodish H, Berk A, Zipursky L, Matsudaira P, Baltimore D. *Molecular Cell Biology*. New York: W. H. Freeman; 2000.

8. Alberts B, Johnson A, Lewis J, Raff M, Roberts K. *Molecular Biology of the Cell*. New York: Garland Science; 2002.

9. Sakai K, Higuchi K, Yamaguchi T, Uchida M. Oxytocin-induced Ca-free contraction of rat uterine smooth muscle: effects of preincubation with EGTA and drugs. *Gen Pharmacol*. 1982;13(5):393–400.

10. Criswell KA, Loch-Caruso R. Lindane-induced inhibition of spontaneous contractions of pregnant rat uterus. *Reprod Toxicol*. 1999;13(6):481–90.

11. Rizzo A, Angioni S, Spedicato M, Minoia G, Mutinati M, Trisolini C, et al. Uterine contractility is strongly influenced by steroids and glucose metabolism: An in vitro study on bovine myometrium. *Gynecol Endocrinol*. 2011;27(9):636–40.

12. Webb RC. Smooth muscle contraction and relaxation. *Adv Physiol Educ*.

2003;27(4):201–6.

13. Arrowsmith S, Keov P, Muttenthaler M, Gruber CW. Contractility measurements of human uterine smooth muscle to aid drug development. *J Vis Exp*. 2018;2018(131):1–12.

14. Smith R. Parturition. *N Engl J Med*. 2007;356(3):271–83.

15. Thornburn GD, Challis JRG. Endocrine control of parturition. *Physiol Rev*. 1979;59(4):863–918.

16. Moir C. Uterine Contraction. *Lancet*. 1935;225(5810):53–4.

17. Bulbul A, Yağci A, Altunbaş K, Sevimli A, Celik HA, Karadeniz A, et al. The role of nitric oxide in the effects of ovarian steroids on spontaneous myometrial contractility in rats. *Theriogenology*. 2007;68(8):1156–68.

18. Weiss G. Endocrinology of Parturition. *J Clin Endocrinol Metab*. 2000;85(12):4421–5.

19. Kawarabayashi T, Kobayashi M, Akahane M, Ajisawa Y. Comparison of in vitro and in vivo inhibitory effects of peptide and nonpeptide oxytocin antagonists on radioligand binding and uterine contractility of rats during pregnancy. *Am J Obstet Gynecol*. 1996;175(5):1348–55.

20. Pehlivanoglu B, Bayrak S, Dogan M. A close look at the contraction and relaxation of the myometrium; the role of calcium. *J Turkish Ger Gynecol Assoc*. 2013;14(4):230–4.

21. McEvoy A, Tetrokalashvili M. Physiology, Pregnancy Contractions. StatPearls Publishing; 2021.

22. Arrowsmith S, Wray S. Oxytocin: Its mechanism of action and receptor signalling in the myometrium. *J Neuroendocrinol*. 2014;26(6):356–69.

23. Chaves VE, Tilelli CQ, Brito NA, Brito MN. Role of oxytocin in energy metabolism. *Peptides*. 2013;45:9–14.

24. Hamid HY, Abu Z, Zakaria B. Reproductive characteristic of the female laboratory

rat. *African J Biotechnol*. 2013;12(19):2510–4.

25. Westwood FR. The Female Rat Reproductive Cycle: A Practical Histological Guide to Staging. *Toxicol Pathol*. 2008;36(3):375–84.

26. Mondal M, Sarkar K, Nath PP, Paul G. Monosodium glutamate suppresses the female reproductive function by impairing the functions of ovary and uterus in rat. *Environ Toxicol*. 2018;33(2):198–208.

27. Miller AE, Riegle GD. Temporal changes in serum progesterone in aging female rats. *Endocrinology*. 1980;106(5):1579–83.

28. Aydin HR, Turgut H, Kurt A, Sahan R, Kalkan OF, Eren H, et al. Ivabradine inhibits carbachol-induced contractions of isolated rat urinary bladder. *Adv Clin Exp Med*. 2018;27(7):893–7.

29. Nishimoto T, Latifpour J, Wheeler MA, Yoshida M, Weiss RM. Age-dependent Alterations in beta-adrenergic Responsiveness of Rat Detrusor Smooth Muscle. *J Urol*. 1995;153(5):1701–5.

30. Semerciöz A, Onur R, Ayar A, Orhan I. The inhibitory role of melatonin on isolated guinea-pig urinary bladder: An endogenous hormone effect. *BJU Int*. 2004;94(9):1373–6.

31. Rajasekaran M, Mehta N, Baquir A, Kuntz S. Rho-Kinase Inhibition Suppresses Potassium Chloride-Induced Bladder Hyperactivity in a Rat Model. *Urology*. 2007;69(4):791–4.

32. Wedenberg K, Ronquist G, Waldenström A, Ulmsten U. Low energy charge in human uterine muscle. *BBA - Gen Subj*. 1990;1033(1):31–4.

33. Steingrímsdóttir T, Ronquist G, Ulmsten U, Waldenström A. Different energy metabolite pattern between uterine smooth muscle and striated rectus muscle in term pregnant women. *Eur J Obstet Gynecol*. 1995;62(2):241–5.

34. Steingrímsdóttir T, Ericsson A, Franck A, Waldenström A, Ulmsten U, Ronquist G. Human uterine smooth muscle exhibits a very low phosphocreatine/ATP ratio as assessed by in vitro and in vivo measurements. *Eur J Clin Invest*. 1997;27(9):743–9.

35. Jespersen B, Tykocki NR, Watts SW, Cobbett PJ. Measurement of smooth muscle function in the isolated tissue bath-applications to pharmacology research. *J Vis Exp*. 2015;(95):1–9.

36. Ayar A, Kutlu S, Yilmaz B, Kelestimur H. Melatonin inhibits spontaneous and oxytocin-induced contractions of rat myometrium in vitro. *Neuroendocrinol Lett*. 2001;22(3):199–207.

37. Edwards G, Henshaw M, Miller M, Weston AH. Comparison of the effects of several potassium-channel openers on rat bladder and rat portal vein in vitro. *Br J Pharmacol*. 1991;102(3):679–86.

APPENDIX: ETHICAL APPROVAL FORM

Protokol No	Toplantı Tarihi	Toplantı Sayısı	Karar No	Proje Yürütücüsü
2019-804	08.11.2019	2019/11	2019/11-3	Prof. Dr. Mustafa Özilgen
‘Thermodynamic Aspects of Age Dependent Deterioration of Myometrium and Detrusor Muscles ’ isimli proje oy birliğiyle etik açıdan uygun görülmüştür.				

Hayvan Türü / Irkı	Toplam Hayvan Sayısı	Hayvanın Cinsiyeti
Sıçan / Sprague dawley	15	Dişi

Görevi	Adı Soyadı	Katılım Durumu
Başkan	Prof. Dr. Bayram YILMAZ	
Başkan Vekili	Prof. Dr. Erdem YEŞİLADA	
Üye	Veteriner Hekim Engin SÜMER	
Üye	Prof. Dr. M. Ece GENÇ	
Üye	Prof. Dr. Rukset ATTAR	
Üye	Prof. Dr. Gamze TORUN KÖSE	
Üye	Doç. Dr. Ediz DENİZ	
Üye	Doç. Dr. Aylin YABA UÇAR	
Üye	Hakan GÖKSEL	
Üye	Ahmet ŞENKARDEŞLER	