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Review Article

Uterine Quiescence in Pregnancy and Labour

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Abstract

The function and regulation of the uterine myometrium are central to pregnancy and labour. Understanding how the contractile unit functions is essential to fully understand how this is manipulated endocrinologically, to maintain a quiescent uterus during pregnancy and then switch to a highly contractile organ at onset of labour. Central to these processes are progesterone which maintains quiescence during pregnancy and corticotrophin releasing hormone which is essential to switching to contractility. When this is triggered too early it leads to preterm labour and all its attendant complications. In this review, I attempt to present a critical review of the abovementioned changes and of the methods that can be employed to predict and delay preterm labour.

Keywords: *Uterine Quiescence; Pregnancy; Labour*

Introduction

The uterus is part of the female reproductive system and is capable of various changes to accommodate a growing fetus and its eventual expulsion. This is accomplished via a number of interactions between different hormones, cells and tissues. Central to all this is the contractile unit made of the myometrial smooth muscle cell which changes its functions according to the stage of pregnancy. In this review, we will see how the basic contractile unit works and how this is manipulated endocrinologically to have a relaxed uterus during pregnancy which maintains the growing fetus, to switch to a state of increased contractility at onset of parturition.

Contractility mechanisms

The myometrial cell

The myometrium is the middle layer of the uterus, sandwiched between the inner endometrium and the outer perimetrium, and is the contractile element of the uterus. It is made of three muscle layers: the inner circular layer, the middle layer made of crisscrossing fibres and the outer longitudinal layer. Smooth muscle cells in the myometrium are supported by a network of connective tissue.

These cells are highly excitable and undergo rhythmic contractions once stimulated by a stimulus. Since a depolarised cell will affect neighbouring cells, the uterus will contract in a co-ordinated way, to push in one direction [1]. This is possible due to the structure of the spindle shaped smooth muscle cells in the myometrium which are linked by gap junctions and thus afford electrical continuity so depolarisation spreads quickly. Actin and myosin filaments are organised in a loose matrix in a way that when the cell contracts it shrinks.

There are also intermediate filaments which connect to the cytoskeleton to aid in transmission of a contraction to the surrounding cells and connective tissue [2].

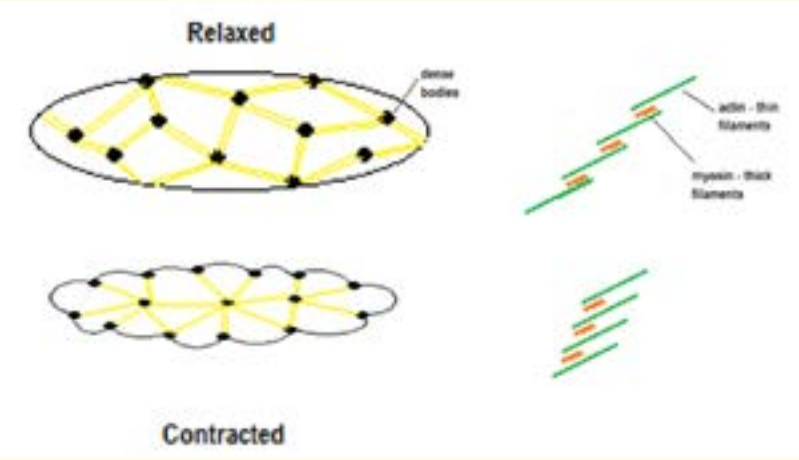

Figure 1: *Myometrial smooth muscle cell.*

The spindle shaped smooth muscle cell is 2 - 5 µm at its widest and 50 - 200 µm long. It is crisscrossed by actin and myosin fibres which are present in a ratio of 10:1 and intermediate filaments which are essential to transmit the force to adjacent muscle cells and connective tissue. These filaments of contractile proteins are anchored to dense bodies in the cytoplasm so that on contraction the cell shrinks as shown above [1].

Excitation-contraction coupling

Actin and myosin are the basic units of the contractile apparatus. Myosin is the heavy chain and is composed of two chains winding around each other to form a tail and two heads. The heads encompass binding sites for actin and ATPase [3]. On the other hand, actin is made of thin, flexible monofilaments about 7 nm wide and is quite a few micrometers long [4]. Uterine myosin hydrolyses ATP to generate energy, however, unlike in skeletal muscle this happens at a slower pace. This is accelerated in the presence of actin.

The first step in launching of the contractile apparatus is the activation of the actin-activated myosin ATPase and this depends on intracellular calcium levels. Calcium enters the cell from extracellular and sarcoplasmic reticulum stores and once inside the cell binds to calmodulin in a ratio of 4:1. The calcium-calmodulin complex then binds to myosin-light-chain kinase (MLCK). The activated MLCK uses ATP to phosphorylate one of the myosin chains attached to the myosin head, leading to a conformational change to allow binding of myosin to actin, followed by movement of the myosin head to shorten the cell [5]. Once the stimulus for contraction ceases, calcium ATPases come into action to remove intracellular calcium. The decrease in intracellular calcium leads to collapse of the calcium-calmodulin complex and thus inactivation of MLCK.

As can be seen, the main molecule which is responsible for linking excitation with contraction is calcium. When at rest, calcium levels are low so as to maintain resting tone. When a contraction is needed calcium levels increase as calcium enters via voltage-gated channels in the cell membrane and sarcoplasmic reticulum membrane [6] and leaves after the contraction to restore resting levels.

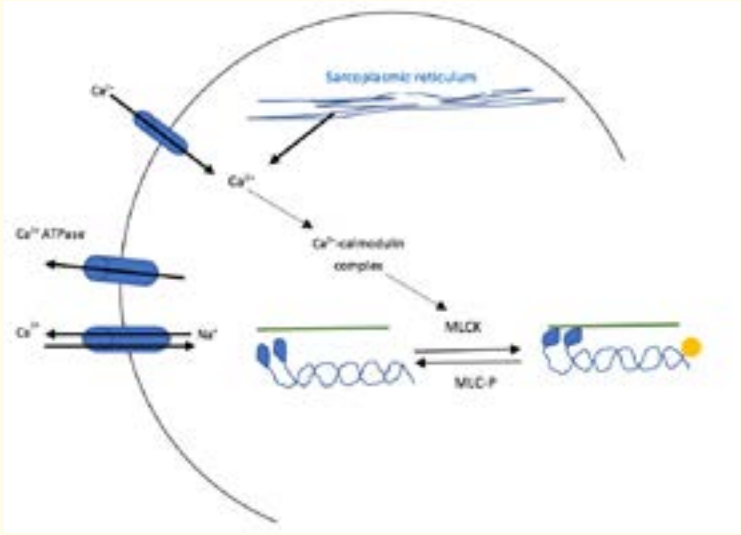

Figure 2: *The control of contractility within the myometrial cell.*

Calcium enters the cell from outside and from the sarcoplasmic reticulum to form the calcium-calmodulin complex which in turn activates MLCK to enact the conformational change in myosin. Actin slides over myosin, leading to contraction. The process is reversed by the action of MLC-P (myosin light chain phosphatase) which comes into play after a few moments to ensure there are no protracted contractions. CaMKII (calcium-calmodulin dependent protein kinase) also phosphorylates MLCK to decrease its affinity to the calcium-calmodulin complex and reverse the reaction. Calcium is extruded from the cell by calcium ATPase and via a calcium-sodium ion exchanger [1,5].

Calcium is obtained from intracellular and extracellular stores - the sarcoplasmic reticulum has inositol trisphosphate (IP3) induced receptors which are triggered after uterine agonists bind to G-protein coupled receptors which activate phospholipase C leading to the production of IP3. There is also calcium-induced calcium release where the rising concentration of calcium within the myometrium sensitises other calcium receptors to open, leading to a positive feedback loop. However, this method, although possible, is still being debated as a mode of increasing intracellular calcium in humans, as to date experiments have been *in vitro* or on animal models [7].

However, intracellular stores are limited and easily exhausted, so there are voltage gated calcium channels on the cell membrane which open when the intracellular voltage changes with release of calcium from the sarcoplasmic reticulum. Other receptors are involved with calcium extrusion, including calcium ATPases which are responsible for maintaining a resting calcium gradient. There are also Na^+-Ca^{2+} exchangers which have a lower affinity to calcium but are triggered by high calcium concentrations. These depend on a sodium gradient which is maintained by the Na^+/K^+ ATPases [8].

The whole process can be fine-tuned by interaction with several other molecules. Actin binds to caldesmon and tropomyosin - molecules which are involved in the regulation of myosin interaction with ATPase - tropomyosin increases ATPase function 30-fold whilst caldesmon decreases its function by up to 80%. In fact, caldesmon is known to increase with stretch to inhibit contractions and thus is essential in the timing of labour. Calponin is another regulatory protein which inhibits the myosin ATPase and thus stabilises the actin cytoskeleton [9].

Molecules from the Ras family, such as RhoA and Ras-1, are essential in the regulation of the assembly of actin and thus affect cell shape and the force generated on contraction. RhoA regulates the action of myosin regulatory light chain phosphatase which dephosphorylates the regulatory light chain of myosin to stop a contraction. *In vitro* studies have also shown that inhibition could be carried out via interaction of Rho with protein kinase N [10]. Further studies have shown that the *in vivo* equivalent of protein kinase N is the myosin-binding subunit of MLC-P (myosin light chain phosphatase) whose function decreases with phosphorylation, leading to an increase in contractility [11].

The uterine pacemaker

The uterus is a myogenic organ as it can contract without neural or endocrine input, however, the site of origin of the stimulus is still debatable. To date there have been no studies that found evidence for a fixed pacemaker site and some believe that unprompted electrical conduction is an inherent property of myometrial cells [12]. Others believe that there are a number of pacemakers in the uterine wall [13] which are generating impulses leading to a directional contraction - these contractions are stimulated by stretch of the uterus and are initially random but with time become synchronised possibly due to the increase in gap junctions [14].

A study done in 2005 showed cells which were morphologically different from myometrial cells that were noted to have numerous projections which connected with surrounding myometrial cells via gap junctions. It is still questionable if these cells can generate an impulse, although once stimulated they generated an outward pulse, and it is thought that they can have a regulatory role in uterine contraction [13]. Studies over guinea and rat uteri showed that contractions do not originate from a single area, although in rats they are more likely to originate from the ovarian horn and occurred at a regular rate. This was not seen in the guinea uterus, although in both species contractions were originating from the mesometrial border [15]. These could represent two different mechanisms of contraction: in one contractions are likely to be originating from the ovarian end and spreading outwards and downwards whilst in the latter it seems that an area is being depolarised, possibly secondary to stretch, and this affects neighbouring cells leading to global synchronisation. Further studies are needed to establish how this mechanism works as both are plausible conclusions.

Gap junctions

Gap junctions are crucial for signal transduction within the uterus as they allow the quick passage of ions, proteins and secondary messengers. They are also essential for electrical coupling of contractions during labour. This is possible as they link the cytoplasm of two adjacent cells bringing them as near as 10 nm within each other [16].

A gap junction is made of protein units called connexins, which join to form an aqueous channel. There are about 21 different connexins which are expressed differently in various tissues and can be assembled in several different combinations. A connexin is made of four transmembrane domains forming the pore, two extracellular loops which are important for cell recognition, an N-terminal which is the same in all types and a variable C-terminus which undergoes post-translational modification. Their assembly into a hemichannel is tightly controlled and is carried out within the sarcoplasmic reticulum and the Golgi apparatus, after which they are packaged into vesicles to be released at the cell membrane. Once they fuse to the membrane they can, either release molecules into the extracellular space, or else aggregate at a particular area in the membrane to form a plaque and associate with another hemichannel to communicate with neighbouring cells. Once their function is done, they are internalised and broken done by lysosomes [17,18].

Connexins 40, 43 and 45 are expressed in the uterus each giving different properties to the junction. They are present in small numbers during pregnancy but, formation of gap junctions was found to increase during labour in response to hormonal stimuli, such as oxytocin and prostaglandins. This is regulated at the level of mRNA and gap junction assembly at the cell membrane. Once labour is over, there is a noticeable downregulation of gap junctions, as they are no longer needed for synchronous contractions [19].

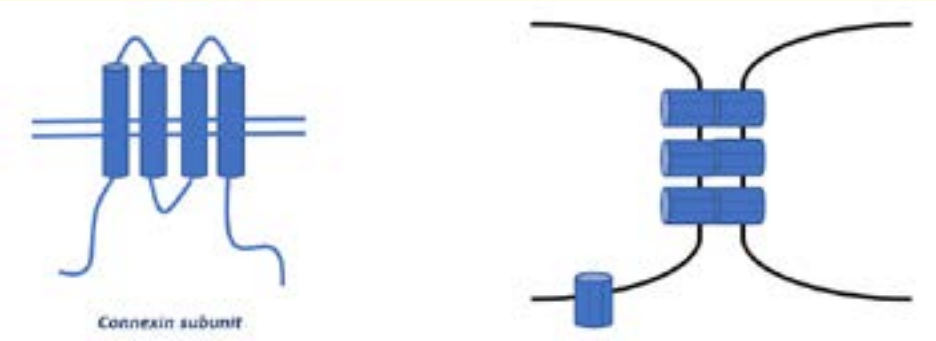

Figure 3: *Gap junctions.*

Gap junctions are made of six protein subunits called connexins, which are in turn made from four transmembrane domains, two extracellular loops and an N and C terminus. They can either aggregate at the membrane and communicate with nearby cells or open into the extracellular space.

Maintaining quiescence during pregnancy

Maintaining quiescence is essential for a successful outcome of the pregnancy. During this stage the myometrium undergoes many changes since it needs to be strong enough to drive the fetus out. It increases in size from about 50g to 1100g due to smooth muscle cell hyperplasia in the initial stages of pregnancy and later by hypertrophy induced by the stretching mechanism. It also expresses different receptors, such as the progesterone, CRH and relaxin receptors, which are essential in keeping the uterus relaxed. From the initial stages of pregnancy till the end, the mother is responsible for fetal wellbeing. During this stage uterine contractions are inhibited to allow the developing fetus to mature fully prior to delivery.

Progesterone

Progesterone is the major hormone of pregnancy and numerous studies have linked elevated levels of it to the maintenance of pregnancy. It binds to the progesterone receptor (PR) which then leads to a cascade of events. The PR can achieve its action by affecting genomic pathways or signalling cascades within the cell. In the former it can affect them directly by affecting transcription or indirectly by acting on ligands which will in turn activate cellular molecules which will affect transcription. On the other hand, the cascade pathways will give a quicker response as they do not involve protein synthesis and can also accomplish the effect by acting on other receptors like the steroid hormone receptors [20].

There are also different isoforms of the PR - these are PR-A and PR-B. Initially it was thought that all progesterone target tissues express PR-A and PR-B with various studies showing that on exposure to progesterone PR-B leads to transcription whilst PR-A leads to minimal transcription. Such observations led to the conclusion that PR-B mediated progesterone's actions by increasing transcription whilst PR-A functions as an endogenous repressor [21]. PR-A has been shown to be transcriptionally active at gene promoters unaffected by PR-B, and studies in mice lacking either one or the other receptor isoform have shown that both receptors have different functions [22]. The same study also showed that the response of reproductive organs to progesterone involves the response of both receptor isoforms. Thus, the effect of progesterone depends on the ratio between the two: when it favours PR-B it is mainly anti-inflammatory with increased production of I-κβα and inhibits proinflammatory gene expression. When PR-A is higher there is more proinflammatory gene expression.

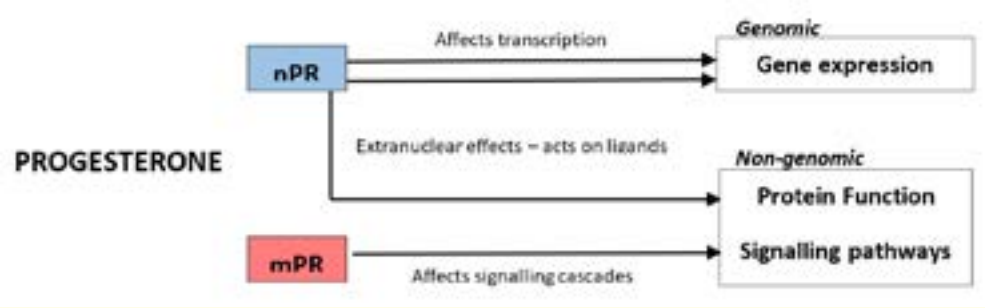

Figure 4: *Progesterone signalling pathways.*

Progesterone has nuclear (nPR) and membrane receptors (mPR) which when activated have various effects. The nPR can lead to changes in transcription of the nuclear contents via the classical pathway as well as by affecting other ligands which in turn have an effect on the nucleus. The membrane receptors (mPR) will affect signalling cascades for quicker results.

Progesterone is essential to modulate the inflammatory mediators associated with labour and decreases the expression of contractile proteins in the uterus and cervix. It is also involved in blocking the oestrogenic effect on production of chemokines which attract immune cells [23]. It mainly modulates the immune response by inhibiting NF-κβ and the ensuing induction of COX-2 and thus the production of prostaglandins. This is achieved via various ways, one of which involves the production of progesterone induced blocking factor (PIBF) from lymphocytes, which adjusts the cytokine equilibrium by inhibiting the release of arachidonic acid and inhibiting natural killer cell activity - in fact, lower levels of PIBF are noted in preterm deliveries [24]. NF-κβ is a molecule which affects the regulation of a significant number of genes, some of which include pro-inflammatory mediators. It is upregulated at onset of labour and is responsible for upregulating contractile associated proteins (CAP); progesterone in turn upregulates the expression of NF-κβ inhibitors such as Iκβ in the uterus and inhibits the activation of NF-κβ by IL-1 [25]. This state of quiescence is maintained as progesterone blocks the IL-1 mediated breakdown of the inhibitor Iκβ via the proteosome pathway.

Progesterone was also found to contribute to delaying preterm delivery due to chorioamnionitis. Samples taken from affected pregnancies show a rise in inflammatory mediators which are chemotactic to neutrophils, a rich source of membrane metalloproteinases (MMPs) which are known to contribute to membrane rupture since they degrade collagen and other ECM components. Progesterone was found to decrease MMP in such cases and could be used in the prevention of premature pre-labour rupture of membranes [26].

Nitric oxide

The production of nitric oxide (NO) is upregulated during pregnancy, and downregulated at term reflecting its ability to inhibit contractility in the uterus. In fact, during pregnancy, uterine muscle is more receptive to NO and in some cases of threatened preterm labour it can be used to delay the onset of labour, with one study quoting a success rate of 74% after administration of transdermal nitroglycerin. However, admittedly it is not used routinely and a systematic analysis concluded that there is insufficient evidence to date for it to be used regularly [27]. On the other hand, other studies have shown that raised levels of NO, in the presence of anaemia, could lead to atony and post-partum haemorrhage [28].

The way NO achieves relaxation is still debatable. It is produced by the action of cytokine-inducible NO-synthase (iNOS) which is upregulated during pregnancy in response to progesterone and cytokines like TNFα and CSF-1. Several studies have shown that NO is

generated within the uterus and it upregulates cGMP leading to a decrease in contractility in human uterine strips in the lab. Additionally, myometrial strips treated with oxytocin exhibited contractility which was decreased on administration of NO [29]. Finally, it was shown that the activity of NO synthase decreases at term, even in cases of preterm labour, indicating that it may have a role in maintaining uterine quiescence.

Relaxin

Relaxin is a polypeptide hormone from the IGF family which is produced by the corpus luteum, decidua and placenta. It has been proven to be a myometrial relaxant in studies conducted on myometrial stripes, with the effect being amplified in the presence of progesterone [30]. In animal models it enables relaxation by upregulating NOS and thus potentiating the effects of NO. It also decreases intracellular calcium and increases cAMP leading to a fall in activity of MLCK. It is essential that its levels are kept at a balance since hyperrelaxinaemia can lead to prematurity because it has a ripening effect on the cervix by upregulating MMPs [31]. Hyporelaxinaemia increases the risk of gestational diabetes [32]. In all, to date most studies have been conducted *in vitro* and thus the effect that relaxin has on the myometrium needs to be studied more before further conclusions can be reached.

Corticotrophin releasing hormone

CRH is released by the placenta and progressively increases throughout pregnancy acting as a clock - in fact women delivering prematurely were found to have higher mid-pregnancy levels. This will cross to the maternal circulation and interact with receptors on the myometrium. There are two forms of CRH receptors - R1 and R2 - each with different variants that are due to different splicing of exons. Different variants are expressed at diverse stages of pregnancy, at different areas of the uterus, leading to varying effects. During pregnancy, the CRH receptor is coupled with the adenylyl cyclase signalling pathway with encourages quiescence by inhibiting MLCK. It can also upregulate iNOS via cGMP synthesis by guanylate cyclase through the action of CRH-R1 to induce quiescence [33].

At onset of labour there is upregulation of oxytocin receptors which leads to release of diacylglycerol and eventual activation of protein kinase C, which in turn desensitises the CRH receptor, decreasing its efficiency. Oxytocin also downregulates guanylyl cyclase leading to less cGMP and loss of its relaxatory effect. It is thought that different receptor isoforms with different functions are expressed at term, thus explaining the dual role of CRH in pregnancy, for example there is upregulation of the R1 subtype during labour [34,35].

Parathyroid hormone-related peptide

Parathyroid hormone-related peptide (PHrP) is one of the local hormones responsible for keeping the uterus in a relaxed state, by increasing placental calcium transport and inhibiting uterine contractility. It also decreases the expression of connexin 43 and oxytocin, working in tandem with progesterone [36]. This effect was found to decrease as the pregnancy progresses, until it disappears at term. It is also hypothesised that it is released in response to uterine stretch in a positive feedback loop.

Labour

Timing of the onset of labour is dependent on several complex hormonal interactions between the maternal and fetal systems, which in turn will lead to the co-ordination of myometrial contractions. The uterus changes from quiescent and expanding to house the growing fetus to a contractile and excitable organ which contracts rhythmically to eject the fetus and placenta. In fact, whilst during pregnancy hormonal control is mainly via negative feedback, during labour there are a number of positive feedback loops where the stimulus for initiation of labour is amplified and the loop is only broken upon delivery.

Fetal factors

The fetus contributes to the timing of birth since it releases several signalling molecules which signals to the mother its readiness by triggering inflammation and eventual delivery.

Cortisol

Fetal cortisol released from the fetal adrenal glands is thought to contribute to parturition. CRH is released from the hypothalamus and the placenta and it activates the maternal and fetal hypothalamic-pituitary-adrenal axis - in the fetal system it acts via a positive feedback loop. This in turn leads to an increase in ACTH which leads to an increased production of the C19 oestrogen precursors DHEA and DHEAS. In humans, this is processed in the fetal liver to be converted to 16-hydroxyDHEAS which is then processed by the placenta to be converted to oestrogen, estradiol and estriol. In fact, CRH was found to increase the mRNA encoding 17α-hydroxylase 17,20-lyase, which is the enzyme needed for the conversion of DHEAS to oestrogen [37].

Fetal cortisol is also essential to increase the expression of receptors for molecules which stimulate contractions like oxytocin, prostaglandins and CRH. It is also important to aid fetal lung maturation by increasing surfactant production which in turn acts as a signal for initiation of labour.

Placental corticotrophin releasing hormone

The human placenta can produce CRH, although it cannot synthesise C19 steroids since it lacks the enzyme CYP17. CRH is an important stimulus to labour since it leads to the production of ACTH from the fetal adrenals and eventual increase in estrogens as explained above. In fact, within the last three weeks of gestation there is a decrease in CRH binding protein in maternal circulation which frees up more of the hormone. Cortisol from the fetus also leads to a positive feedback loop to up the production of oestrogens since it stimulates the placenta to release more CRH, amplifying its effect.

The high levels of CRH at term also aid the production of CAP by increasing expression of connexin-43 by activating the nuclear transcription factor activator protein-1 (AP-1). AP-1 in turn activates other cytokines and stimuli leading to contractions [38]. Other evidence that CRH is the timer to labour is that it increases the response of the myometrium to prostaglandin F2α and activates IL-1 so that it prepares the myometrium to other inflammatory cytokines [39]. It is also involved in progesterone withdrawal since experiments on placental trophoblast have shown that CRH decreases the enzymes responsible for progesterone synthesis via a PKS-dependent pathway - there is a decrease in cytochrome P450scc which changes cholesterol to pregnenolone and HSD3b1 which converts pregnenolone to progesterone.

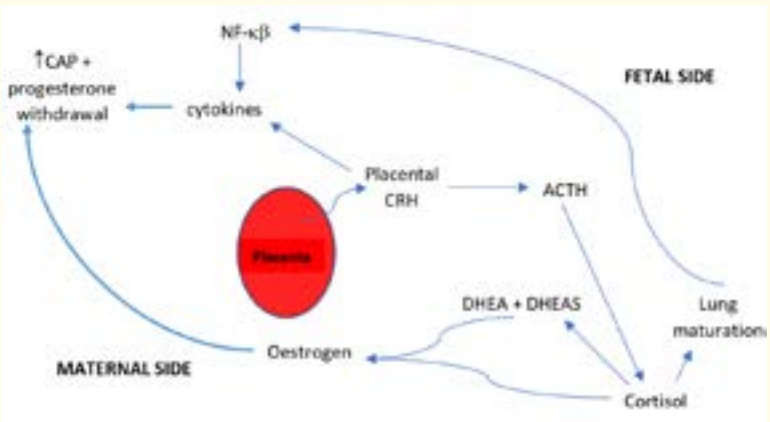

Figure 5: *Summary of the effects of CRH in parturition.*

Placental CRH causes the release of ACTH in the fetus which stimulates the release of oestrogen precursors in the fetal liver which are then converted in the placenta. It also increases the release of cortisol which boosts fetal lung maturation which in turn increases NF-Kβ in the maternal system to induce the release of cytokines. Cytokines and oestrogen lead to progesterone withdrawal and increased expression of contractile associated proteins (CAP).

CRH acts via interaction with its G-protein coupled receptor which is available in two isoforms - CRH-R1 and CRH-R2 - each with different functions. In strips of non- labouring myometrium CRH-R1 was found to inhibit contractions by inhibiting phosphorylation of MLCK. At term it is more abundant at the lower segment of the uterus and decreases significantly at the fundus which needs to contract. On the other hand, there is increased expression of CRH-R2 towards term as it increases phosphorylation of MLCK and stimulates contractions [40]. Thus, with these receptor isoforms CRH can have a dual action on uterine contractility.

Surfactant

Lung surfactant is a glycerophospholipid-rich lipoprotein that is produced during the third trimester, after signalling by cortisol stimulates the type II pneumocytes to produce it. It has four proteins: SP-B and SP-C are responsible for decreasing alveolar surface tension whilst SP-A and SP-D stimulate the immune system. All are excreted in the amniotic fluid by the fetus' breathing movements and the latter bind with Toll-like receptors which span the membrane and act as immunoregulators - this will trigger NF-κβ and upregulate cytokines triggering the inflammatory cascade in the uterus leading to uterine contractions [41]. There is also activation of macrophages which migrate to the myometrium leading to higher levels of IL-1 and activation of NF-κβ - this will increase the expression of CAP and oppose the effect of progesterone by interacting with the PR. The activation of NF-κβ will also lead to initiation of the transcription of a number of target genes like COX-2, which catalyses the synthesis of prostanoids leading to increased uterine contractility [42]. In conjunction, surfactant also stimulates the release of prostaglandin E since it acts as a source of arachidonic acid which is a precursor for prostaglandins. In conclusion, the maturing lung is releasing surfactant proteins which are signalling to the uterus that the fetus has developed and is ready for delivery. Thus, the surfactant acts as a hormonal stimulus that causes migration of macrophages to the uterus, triggering the inflammatory cascade to initiate delivery [43].

Maternal factors

The maternal system contributes to the timing of delivery after responding to stimuli from the fetus.

Progesterone withdrawal

Withdrawal of progesterone is a key step in the initiation of labour, however, in humans it was noted that progesterone levels remain elevated throughout the third trimester and labour. In fact, progesterone withdrawal in humans occurs at the molecular level as the progesterone receptor function is impaired via changes in the co-regulators and activation of local inflammatory pathways which lead to changes in the PR isoform [44]. There are three isoforms of the receptor with the PR-B transactivating other genes which accomplish progesterone's function. When expressed with PR-A, the latter will inhibit PR-B as it interacts with co-repressors instead and during delivery PR-A expression is upregulated. On the other hand, PR-C does not have a DNA binding domain but can inhibit progesterone function by segregating progesterone in the cytoplasm unable to bind with PR-B. Studies have shown that on activation of NF-κβ at term there is upregulation of PR-C which in turn impairs the function of PR-B [44].

Another way through which progesterone is withdrawn is via the nuclear progesterone receptors (nPR) and their interaction with the gene promoters. At term there is a reduction of nPR coactivators which leads to a decrease in histone acetylation making the promoter regions unreachable to the nPR transcriptional complex. Without this bonding, progesterone would not be able to complete its function [45].

On withdrawal of progesterone there is also a switch from relaxatory CAP to stimulatory CAP. These CAP include the oxytocin and prostaglandin F2a receptors. This switch also leads to the production of prostaglandins, COX-2 and gap junction proteins, mainly connexin 43 [20]. In fact, studies have shown that there is differential expression of connexin 43 and COX-1 and 2 within the uterus, with connexin 43 being more concentrated at the fundus and COX at the lower segment.

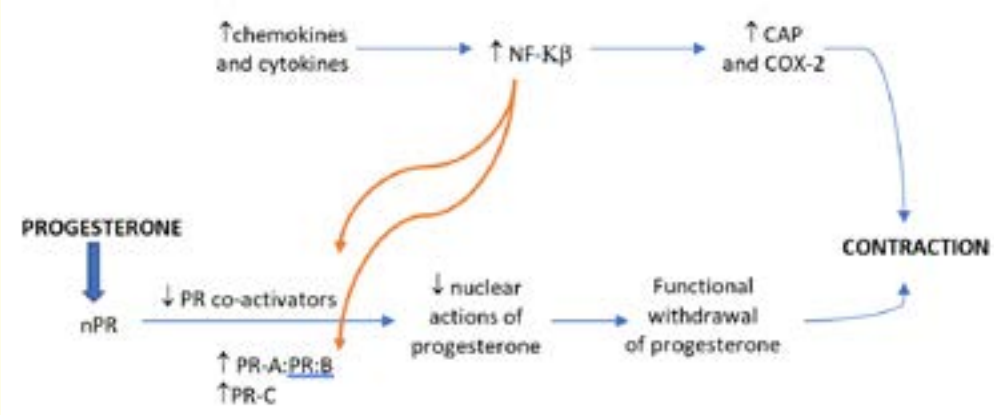

Figure 6: *Progesterone withdrawal.*

At term functional withdrawal of progesterone occurs as the ratio of A:B receptors changes. At the same time the increase in chemokines and cytokines increase NF-Kβ which increases PR-C to inhibit the nuclear functions of progesterone and aids in its withdrawal. There is also increased expression of contractile associated proteins (CAP) which aid in contracting the uterus. This process is also possible due to a fall in the co-activators, because of the rise in NF-Kβ . These co-activators include CREB binding protein and steroid receptor co-activators whose function is to keep DNA acetylated so that genes can be expressed. Thus, with their fall, DNA is condensed and progesterone responsive genes are not expressed.

Oxytocin

Oxytocin is a nanopeptide synthesised in the supraoptic and paraventricular nuclei of the hypothalamus and secreted from the posterior pituitary gland, promoting uterine contractility and the ejection of milk from the breasts. Oestrogen promotes its release whilst progesterone inhibits it. It carries out its function of promoting uterine contractility by activating phospholipase C leading to opening of the calcium channels and eventually contractility. In fact, during labour oxytocin is released in pulses of short duration which progressively increase in frequency as labour progresses [46].

Studies carried out in rats lacking oxytocin showed that they still went into labour as they had a compensatory rise in vasopressin. However, further studies showed that a rise in oxytocin is not that essential in labour since there is upregulation of the receptors within the myometrium at a time when uterine sensitivity is greatest. Thus, lower levels of oxytocin are sufficient to mount a response. Oxytocin is also produced by the placenta and amnion, which in turn also upregulates the oxytocin receptor up to four-fold. Its role in labour is also evidenced by the fact that it is used to augment labour with good effect, whilst its antagonists are used with some effect to delay preterm labour [47]. However, mice lacking oxytocin and women suffering from panhypopituitarism still have normal labour, putting into question its role in the initiation of labour.

Oestrogen

Oestrogen is a pro-contractility hormone towards which sensitivity late in pregnancy increases, and which is produced by the actions of cortisol and CRH as detailed previously. Throughout pregnancy, levels of oestrogen and progesterone, the pro-relaxatory hormone, remain high and it is at the molecular level that oestrogen takes over at term. In fact, oestrogen has a facilitatory function with regards to

contractions since it is essential to increase the concentration of oxytocin and alpha-adrenergic receptors in the myometrium. It is also important for intracellular communication, since at term when the oestrogen progesterone ratio favours oestrogen, there is an increase in gap junctions between cells [48]. By controlling the release of prostaglandins, it also has a role in cervical maturation. Its importance for parturition was demonstrated by experiments on rats, where those who had ovariectomy during pregnancy had a prolonged labour with high fetal mortality rate, despite being administered oxytocin. Incidentally, these experiments also highlighted oestrogen's importance for lactation and for maternal behaviour post-partum.

Inflammatory pathways

Inflammation of the decidua and membranes is a common finding during labour [49,50] as is inflammation of the myometrium, which increases after rupture of membranes. In fact, they are highly correlated and it is difficult to have one without the other [51]. Recently it was found that the presence of neutrophils and macrophages in the myometrium during parturition at term is physiologic and is associated with the process of cervical maturation. Inflammatory cells are more marked in the lower segment and in fact, they even infiltrate the cervical stroma to aid cervical ripening by releasing collagenases which break down collagen and soften the cervix [52]. There is also infiltration of the myometrium and placenta to aid in membrane rupture by secreting proteolytic enzymes and to contribute to the timing of parturition as cytokines in the amniotic fluid trigger the production of prostaglandins. The fact that labour is an inflammatory process is also supported by the fact that there is an increase in expression of adhesion molecules to aid in attachment and extravasation of inflammatory cells - these include E-selectin, ICAM-1 and 2, VCAM-1 and PECAM.

At the initiation of labour there is infiltration of neutrophils and macrophages into the myometrium, especially in the lower segment. The activated cells will in turn recruit other inflammatory mediators such as the cytokines IL-1, IL-6 and IL-8 which break down Iκβ and activate NF-κβ [53]. The activated NF-κβ then activates the expression of genes responsible for the production of proteins essential for contractions, such as connexin 43, oxytocin receptors and COX-2. Additionally, it is thought that NF-κβ also affects contractility indirectly by blocking the effects of progesterone on genes [43]. In the lower segment, other inflammatory mediators like collagenase and elastase are recruited since these are essential for tissue remodelling to aid cervical dilation and eventual expulsion of the fetus.

Numerous inflammatory molecules are involved in the process, one of which is MCP-1. This is a β-chemoattractant which when present in high levels in the uterus is associated with inflammation, as it attracts and activates macrophages. It is increased in the presence of NF-κβ and during labour but is inhibited by progesterone [54]. Thus, as progesterone decreases it increases, leading to inflammation and eventually labour.

Prostaglandins

Prostaglandin metabolism changes throughout pregnancy. There are prostaglandin H synthases (PGHS-1 and PGHS-2) in the amnion which produce prostaglandins, especially at the onset of labour [55] however, there is also prostaglandin dehydrogenase (PGDH) in the chorion which is breaking down prostaglandins to prevent fetal products from reaching the maternal circulation. The balance between these two components is essential for the timing of labour; prostaglandins made in the amnion do not easily cross to the maternal circulation since PGDH is continuously metabolising it. Several studies have confirmed this, however three studies showed that there is some crossing over suggesting that PGDH is not present in the same concentrations throughout the placenta. On rupture of membranes, there is breakdown of the PDGH barrier leading to passage of prostaglandins to the mother and the onset of a cascade leading to onset of labour. When taking sections from the uterus and analysing them, studies in baboons have shown that PGHS is more abundant at the lower segment where prostaglandins are essential for cervical ripening whilst PGDH levels are low at the fundus [56], however, this has yet to be confirmed to occur in humans.

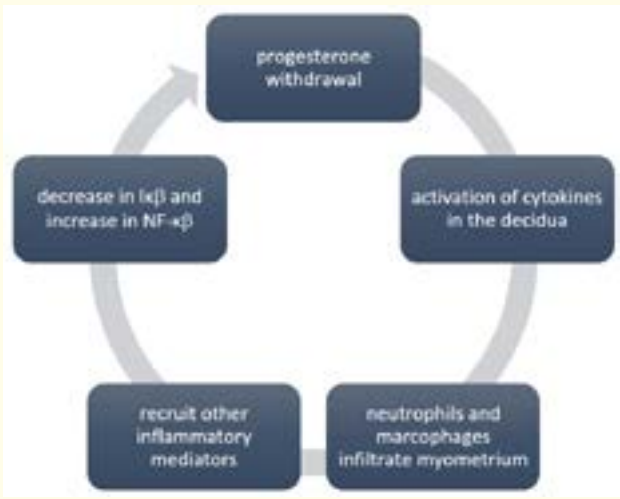

Figure 7: *Inflammation at term.*

At term there is progesterone withdrawal which is initiated as described above. This will stimulate the decidua to release pro-inflammatory cytokines which will in turn attract neutrophils and macrophages to the uterus. Bacteria can also cause a similar reaction. Inflammatory mediators will inhibit Iκβ and activate NF-κβ which will affect gene expression to increase the presence of CAP responsible for contractions and further blocks progesterone function. Cytokines also increase production of MMPs, COX and prostaglandins which are involved with cervical maturation and membrane rupture.

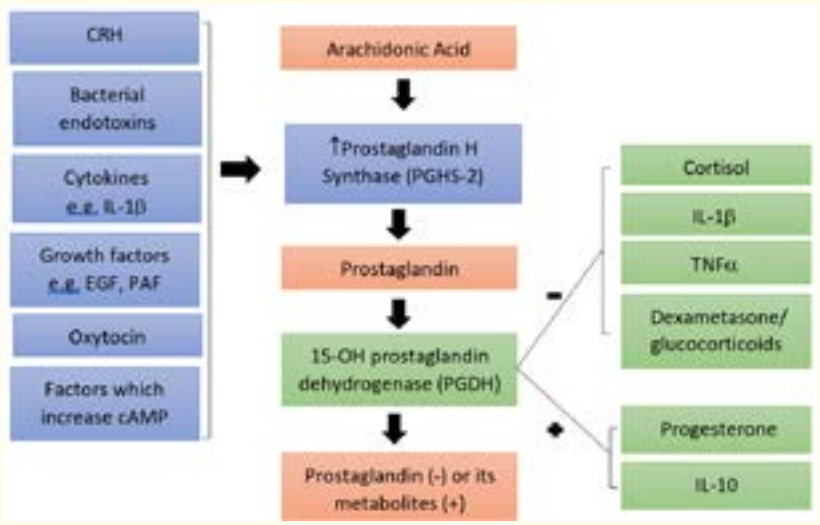

Figure 8: *Summary of prostaglandin metabolism.*

Arachidonic acid is the source for prostaglandins, which are produced by PGHS-1 and PGHS-2. This step can be stimulated by several molecules including IL-1β which increases the transcription of PGHD-2 and stabilises the mRNA of the protein. This is inhibited by the administration of glucocorticoids and is potentiated by IL-10. Other cytokines like p50 and p65 of the NF-κβ also stimulate PGHS function. When stimulated, PGDH breaks down prostaglandins leading to uterine quiescence. When it is inhibited, there is more free prostaglandins which leads to uterine contractility and cervical dilation. Progesterone is produced by the action of 3β-hydroxysteroid dehydrogenase in the chorion, and together with IL-10 promote PDGH activity and by extension quiescence.

The effect that prostaglandins have on uterine quiescence and contractility depend on the balance between synthesis by PGHS and breakdown by PDGH. Studies on preterm labour have shown that there is less PDGH expression which leads to more free prostaglandins to bind to the receptors and the fall in PDGH activity is steeper in preterm labour when compared to those who go into labour at term. Additionally, PGE2 stimulates MMP-9 which is responsible for the degradation collagen and eventual membrane rupture [57]. Progesterone also has a role in maintaining prostaglandin levels low during pregnancy since it maintains PGDH levels.

Prostaglandin levels rise towards term as its release is stimulated by CRH and in turn stimulates production of cortisol from cortisone via the enzyme 11β-hydroxysteroid dehydrogenase-1 leading to a positive feedback loop. The presence of PGE2 and PGF2α enhances cervical ripening and uterine contractility [57]. It is also thought to be important for fetal adaptation to labour by decreasing movements and breathing to conserve energy [58], upregulating the hypothalamic-pituitary-adrenal axis to increase cortisol release and thus contribute to onset of parturition [42], membrane rupture as it promotes the increase in MMP-9 to break down collagen and the maintenance of utero-placental blood flow [42].

Uterine stretch

For the stretch stimulus to be effective at stimulating contractions, there should be the optimal endocrine environment, as high levels of progesterone will inhibit this ability. However, at term stretch induces the expression of connexin-43 and oxytocin receptors. In the initial stages of pregnancy, the high levels of progesterone support uterine hypertrophy as the uterus grows to accommodate the fetus. However, later in pregnancy when progesterone levels fall, the uterus grows less and the tension in the wall increases, triggering an increase in CAP and contractions [42]. For example, studies have shown that stretch increases expression of oxytocin receptors by increasing mRNA production. Another study showed that stretch leads to contractility: during pregnancy there are increased levels of caldesmon which inhibits the ATPase of myosin maintaining quiescence. With progression of pregnancy there is increased stretch which will activate the focal adhesion molecules connecting the cytoskeleton to the extracellular matrix and which are important for mechanical transduction of force. Signalling via ERK will phosphorylate caldesmon and inhibit it [59].

Preterm labour

In preterm delivery, there is asynchrony between fetal development and the onset of labour and it happens in 8 - 10% of pregnancies. Causes could be iatrogenic as labour is induced due to serious fetal or maternal conditions like eclampsia, due to premature rupture of membranes or idiopathic. About 40 - 50% are idiopathic whilst 30 - 40% are due to an underlying infectious process; in fact, pregnancies ending with preterm labour were found to have higher levels of interleukins in the amniotic fluid and more leucocytes in the myometrium which increase cytokine release.

Preterm labour is associated with several lasting effects including mental retardation, neurodevelopmental delays and physical handicap. Thus, it is essential to prevent preterm labour to avoid such repercussions. Understanding the processes underlying its pathology will enable us to manage the condition better.

Predictive methods

Although preterm labour has such a significant impact on neonatal morbidity and mortality, there is little we can do to accurately predict and prevent it. The incidence is increasing in the developing world, although this increase could be partly contributed to the increase in assisted reproduction, a risk factor for preterm delivery. An accurate test would aid in diagnosis and management of such cases, however, to date this is difficult as diagnosis is subjective and the initial symptoms are mild and can be present in a normal ongoing pregnancy.

Risk assessment

A maternal history is the most important tool to assess the risk of preterm birth as there are several factors which increase its likelihood. A large-scale study identified several factors which increase the risk of preterm delivery. These included a young maternal age, ethnicity, low BMI, smoking and previous cervical surgery. When these factors were combined with cervical length measurement, the detection rate went from 38% to 69% [60,61]. A previous preterm delivery is one of the most important predictors, as well a short pregnancy interval, multiple pregnancy and assisted reproduction [62].

Genetics were also found to play a part, with patients whose sister had a preterm delivery nearly having double the risk. A huge review has shown how genetics contribute to the risk of preterm labour - in fact polymorphisms of molecule involved in the inflammation pathway were found to increase the risk, which is further increased by stress and infection [63]. The most commonly studied polymorphisms were for TNFα and IL-6 and in most studies they were found to be associated with an increased risk, however, this is not routinely tested for or treated in clinical practice, although in future testing for such polymorphisms may aid in screening susceptible patients. Infection by bacterial vaginosis also increases the risk of preterm labour, especially in those with TNFα polymorphism, showing how environmental factors can interact with genetic susceptibilities to affect risk [64]. In fact, vaginal infections, including those with enteropharyngeal bacteria [65], are thought to contribute to early onset of labour, however, consensus has not yet been reached on the significance of this and whether these should be treated [66]. What is important to keep in mind is that alone these factors are not causative of preterm labour, however when combined they increase the risk.

Cervical length measurement by ultrasonography

A short cervix could be due to multitude reasons including previous surgery on the cervix, uterine inflammation, progesterone decline, diethylstilbestrol exposure in utero and idiopathic cervical insufficiency [67]. However, more commonly with the onset of labour, the cervix will dilate and efface - these changes will start from before the active onset of labour. Vaginal examination is very subjective; thus, a transvaginal ultrasound is a more accurate, unbiased way of measuring cervical length. Studies show that a short cervix and funnelling before 20 weeks of gestation in patients who had a preterm delivery in the past is predictive. A short cervix in a symptomatic woman is also predictive of a preterm delivery. Although there is no consensus on the cut-off for cervical length, numerous studies quote a length of less than 30mm as predictive of preterm labour [68].

Unfortunately, most studies had small population groups, but a meta-analysis done in 2010 concluded that a cervical length of less than 15 mm in a symptomatic patient will predict about 60% of deliveries occurring within 1 week. A cervical length of 15 mm in singleton pregnancies is only seen in about 10% of symptomatic patients and increases the risk of delivery within 1 week by 5.7 times. Having a negative predictive value of 96% for a cervical length of more than 15mm is also reassuring [69].

In twin pregnancies, the risk of preterm labour is slightly higher with about 20% of cases presenting with tightenings delivering within 7 days, compared with the 10% that would deliver in singleton pregnancies. As a reflection of this, the cut-off cervical length in twin pregnancies if 25 mm [70].

Fetal fibronectin

Fetal fibronectin is a glycoprotein found at the interface between the membranes and decidua which is thought to act as an adhesive between the two. It is absent from cervical fluid from 24 weeks onwards and is thought to be released near term due to mechanical and inflammatory meditated damage to the membranes during labour [71]. It has been shown that its release is enhanced by infection with bacterial vaginosis which also leads to inflammation, and which in turn increases the risk of chorioamnionitis.

Sampling the cervical discharge for this could give an indication if preterm labour is likely to occur within the next 7 - 10 days. However, a systematic analysis showed that it has a high negative predictive value but not a good positive predictive value [72]. Thus, such a test would have limited accuracy in the asymptomatic patient, but is helpful in the symptomatic patient, when assessed within the clinical context and is better at excluding preterm labour rather than predicting it. In fact, a meta-analysis showed that in symptomatic patients it has an 89% sensitivity. Additionally, its relation with cervical length is inversely proportional and the latter was found to be a more accurate way of predicting preterm birth [73].

Endocrine factors

Although biochemical markers are more accurate at predicting preterm labour, the majority are not routinely used in clinical practice due to varying reasons including cost and lack of sufficient studies to justify its use. Placental proteins such as α-fibronectin can be detected in cervical fluid as discussed above, and to date it is the most reliable method of detecting early preterm labour.

One can also detect placental hormones like CRH, prolactin and HCG. Oestrogens and relaxin are other hormones released from elsewhere that can change as labour approaches. Unfortunately, most of these parameters are only useful in detecting late preterm labour which has minor consequences on fetal morbidity and thus does not need treatment [71]. One such example is the detection of salivary oestriol, which rises because of the rise in maternal oestradiol prior to delivery. However, oestriol levels peak at night and can be supressed by betamethasone, leading to a high false positive rate [74].

Preventive measures

Progesterone

When given to patients with a history of preterm birth, the incidence of spontaneous preterm birth is halved. However, it is also beneficial when given to patients who have a short cervical length of 15 mm or less at a 20 - 24 weeks scan [75]. As discussed above progesterone is critical in the maintenance of uterine quiescence and supplementation can prolong that state in high risk pregnancies.

Several meta-analyses concluded that the administration of progesterone aids in decreasing the incidence of preterm labour before 34 weeks - use of 17α-hydroxyprogesterone caproate, vaginal pessaries and oral treatment with progesterone showed similar results. However, all concluded that there needs to be further investigation of the effect on neonatal morbidity [76-78]. On the other hand, some recent studies showed that progesterone has little to no effect on preterm labour [79], thus a consensus has not yet been reached and further clinical trials are needed.

With regards to twin gestations, consensus has also not been reached as different studies have conflicting results. In a randomised, placebo-controlled trial of naturally occurring twin gestations with no history of preterm labour there was no statistical difference between the two groups with regards to gestation at birth [80]. On the other hand, a meta-analysis of two trials done on women pregnant with twins who had a short cervix showed a minimal improvement when compared to the placebo group, as well as less neonatal morbidity. From the evidence it is clear that one has to consider the clinical state of the patient before deciding whether to prescribe progesterone or not.

Cervical cerclage

Cervical cerclage has been the preventive modality most in use for risky pregnancies, since it has been in use for over 50 years. It involves the application of a Shirodkar or McDonald suture around the cervix which will tighten the os and ensure it stays closed until term. It is done under spinal or general anaesthesia and may be associated with some bleeding after the procedure and rarely infection.

A multicentre randomised control trial showed that the use of cervical cerclage in patients with a short cervix does not decrease the incidence of preterm labour or perinatal morbidity. This was also seen other studies as well, which showed that routine cerclage is not beneficial [81]. On the other hand, when applied to high risk patients with a history of second trimester miscarriage or previous preterm birth, it was found to be beneficial [82]. Consensus has not yet been reached with regards to the efficacy of cervical cerclage in twin pregnancies. Thus, it makes sense to offer cervical cerclage to those with a history of previous preterm births or second trimester losses, however, it is to be avoided in those with no history of PTB or in twin gestations.

When compared with vaginal progesterone, an indirect meta-analysis found that both options are useful in the singleton pregnancy with a short mid-trimester cervix in a history of previous preterm birth, and which one is used depends on clinician preference [83]. Similar conclusions were reached when comparing with vaginal progesterone and pessary [84], although clinical trials comparing all three would be ideal to reach a definite conclusion.

Pessary

The silicone pessary is placed transvaginally around the cervix, to support it and change its direction towards the sacrum, thus reducing pressure on it from the uterus and its contents. Several randomised clinical trials have been carried out to test this hypothesis but the results have been inconclusive with different trials having different conclusions [85,86]. The one with nearly 1000 participants showed that it does not decrease the incidence of preterm birth when compared with progesterone [87]. However, there was no blinding in the trial and this might have affected the allocation of patients to treatment groups, with those with a shorter cervical length more likely receiving progesterone. Similar inconclusive results were obtained in twin pregnancies [88].

	Short cervix + no previous PTB	Short cervix + previous PTB	Short cervix + twins
Cerclage	×	✓	×
Progesterone	✓	✓	? ✓
Pessary	Controversial	Controversial	Controversial

Table 1: *Summary of different treatment modalities to prevent preterm birth (PTB).*

Cervical cerclage was found to be beneficial in patients with a history of previous PTB and a short cervix on ultrasound, however, was found to be less beneficial in other population groups [83]. Vaginal progesterone given from 24 to 34 weeks in those with a short cervix, regardless of a history of PTB, was shown to be effective at decreasing the incidence of PTB. In the same study a small amount of twin gestations were included and results in this cohort were inconclusive, possibly due to the small size of the sample [75]. Pessary use in all situations has not been studied enough and to date remains controversial in its use in clinical practice [85-88].

Tocolysis

Tocolytics are used to prolong labour, mainly to given in those with less than 34 weeks gestation to allow enough time for the corticosteroids to enact their effect. However, their use is still controversial as despite their widespread use in preterm labour, their efficacy has not been proven sufficiently.

There are various tocolytic drugs, each with their own benefits and side effects, which can be found summarised in table 2. Taking all the information, the best combination is found in calcium channel blockers and prostaglandin inhibitors [89]. On the other hand, another trial compared nifedipine and atosiban and found them to be similar with regards to delaying labour and perinatal morbidity [90]. All this shows that much more needs to be done before a definite decision on the perfect drug is made, especially as to date there has been no clear evidence that tocolysis helps with perinatal morbidity and mortality.

	Calcium channel blockers E.G. Nifedipine	Magnesium Sulphate	Oxytocin receptor blockers E.G. Atosiban	Prostaglandin inhibitors E.G. Indomethacin	Beta mimetics E.G. Ritodrine, Terbutaline
Delay of labour by 48 hours	3	2	5	1	4
Neonatal morbidity	1	5	4	2	3
Respiratory distress syndrome	Results from the meta-analysis show that there is no major difference between the different classes with regards to their effect on RDS.				
Maternal side effects	3	4	2	1	5

Table 2: *Summary of effect of different tocolytic drugs.*

To date no trial has been done that compares all the possible treatment options, so till then meta-analyses must suffice. All the drugs will delay labour more than a placebo and their efficacy is rated as above. With regards to the prevention of neonatal morbidity, the evidence is uncertain, however the fact that they allow enough time for corticosteroids to work is beneficial, as the latter with aid with lung maturation and decrease respiratory complications [89].

Conclusion

In conclusion, one can see that the process by which the uterus converts from quiescence to contractility is complicated and much more needs to be done to properly understand how this is comes about. Understanding this complex process is essential if we are to be able to help mothers presenting with preterm labour and decrease the perinatal morbidity and mortality that ensues from such early births.

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