The Uterus - a Progesterone Target Organ

ANDA GHEORGHITA[1], DRAGOS NEMESCU[1,2]*, ELENA MIHALCEANU[1,2] , ALINA PLESEA CONDRATOVICI[3], MIRCEA ONOFRIESCU[1,2]

[1] Cuza Voda Clinical Hospital of Obstetrics and Gynaecology Iasi, 34 Cuza Voda Str., 700038, Iasi, Romania

[2] Grigore T. Popa UMF Iasi, Department of Obstetrics and Gynaecology, 16 Universitatii Str., 700115, Iasi, Romania

[3] Dunarea de Jos University of Galati, 47 Domneasca Str., 800008, Galati, Romania

Progesterone is a steroid hormone synthetized from pregnenolone, a derivate of cholesterol, produced by the yellow body of the ovary and by the placenta during pregnancy. It is crucial for the evolution of the pregnancy, from preconception until delivery. It prepares the endometrium for implantation, supresses the immune response, inhibits the myometrium's contractility and it can also make the myometrium sensitive to the action of beta-mimetic tocolytics. The vaginal administration of progesterone in various doses has proved effective in preventing premature birth in pregnant women with short cervix ≤ 25mm during transvaginal ultrasound exam.

Keywords: Progesterone, embryo implantation, yellow body, placenta, premature birth

During pregnancy, the uterus fundamentally changes its anatomic and histological structure as well as its functional capacity. The transformations which uterus undergoes during pregnancy influence all structural elements of muscular, conjunctive and vasculo-nervous tissue so to ensure the attachment of the egg, the development of the foetus and the expulsion of the product of conception [1].

The uterus consists of three distinct layers: the inner layer: endometrium, that protects the lumen of the uterus, the middle layer: myometrium and the outer layer: serosa. The endometrium includes the basal layer that is permanent and adjacent to the myometrium, and it has a regenerative role and the functional layer, with a relatively short life span covering a menstrual cycle.

Initially, in the follicular phase, the endometrium experiences different proliferation processes of the glands, stroma and vessels, and subsequently, the apparition of progesterone alongside with the formation of the corpus luteum, in the secretory phase generates stromal and glandular modifications. They lead to synthesis and distribution of glycogen deposits into the glandular system, stromal oedema, supporting the development of the arteriolar system, all for preparing the endometrium for the implantation of the blastocyst during the middle luteal phase. In case of fecundation and implantation, the proliferated endometrium that subsequently presents secretory modifications undergoes decidualization [2].

The myometrium represents the contractile element of the uterus, consisting of smooth muscular fibres; their orientation and distribution on layers has been highly disputed. Presently, it is considered a three layer distribution, the outer layer being the best represented one; it consists of a web of muscular nods that frequently surround the vascular channels; they play an important role in gestation contractility, delivery and in the immediate post-partum period; the inner layer has numerous estrogen and progesterone receptors regulated during the menstrual cycle, important in the contractility of non-pregnant uterus and the third layer, subendometrial [3].

To protect the product of conception and to ensure its development until term, the pregnant uterus increases its volume constantly, ensuring a permanent parallelism between the growing content and the carrier. The uterus compliance is ensured through hypertrophy, hyperplasia and metaplasia.

Body and isthmus musculature increase by 70% during pregnancy and since the uterine surface undergoes a more rapid increase compared to the uterine musculature, this leads to a relative diminution of the uterine wall thickness.

In the first pregnancy trimester, the HCG (human chorionic gonadotropin) secreted by the gestational trophoblast maintains the yellow body's capacity to synthetize progesterone, until the placenta comes into action. This is the major endocrine organ during pregnancy, the function being regulated by receptors through endo-, para-, auto- mechanisms [4].

Syncytiotrophoblast (ST) and cytotrophoblast (CT) are the key regions where hormones are produced, where ST synthetizes HCG and steroids. The progesterone secreted in the placenta will go both to the mother where it will be metabolized and excreted by the kidneys and to the foetal organism [5].

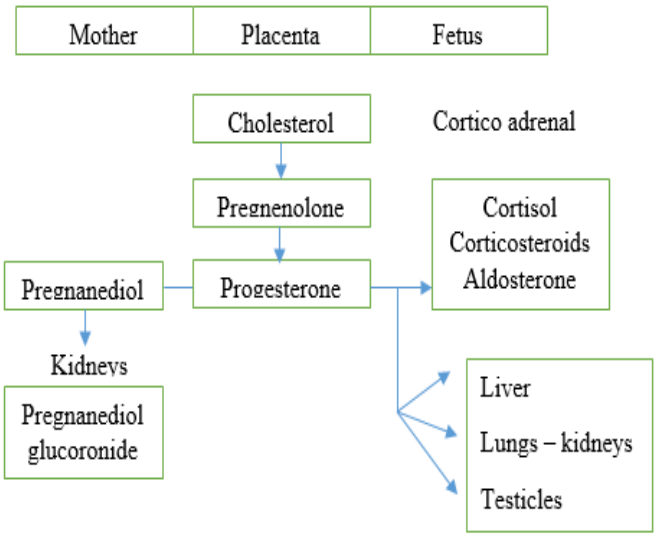

Fig. 1 Progesterone biosynthesis (modified from Merger) [5]

* *email: dragos.nemescu@umfiasi.ro*

Data confirming the existence of placental progesterone [6]

In humans, just like in other mammals, the ovaries can be extirpated in the last stage of pregnancy (when the yellow body normally goes into regression), without interrupting the gravidity state;

If in female rats, where the yellow body persists naturally until the end of the gestation, all cubs are removed except for one, keeping the placentas, gestation continues uninterrupted even after ovariectomy

Collip et al. also found that in female rats, ovariectomy and the extirpation of all cubs maintaining the placenta intact, did not interrupt the characteristic development of the endometrium during pregnancy;

In female cats, in the first stage of pregnancy, ovariectomy causes the death of embryos but the placentas continue developing, and the progestative activity of the endometrium continues progressing.

The chemistry of progesterone

The active principle of the yellow body was obtained in crystalized form. The crystalized product named progesterone can transform itself into a thermostable form ($\Delta_f H$ = 26.17 kJ/m). The previous name of progestin was maintained for the non-purified, lutein principle that common yellow body extracts have. Progesterone presents itself as two isomeric forms: α- and β-progesterone. Their physiological action is almost equal.

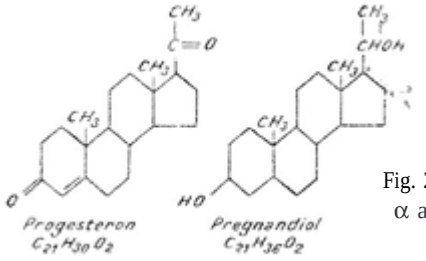

Fig. 2. Isomeric forms of α and β-progesterone

Starting from sterol (stigmasterol) obtained from soya beans, Butenandt et al. [7] have managed to reach the last stages of the synthesis of progesterone, preparing it from pregnanediol, as well as from cholesterol. Block also noticed that if a pregnant woman ingests isotope-marked cholesterol, this leads to the apparition of pregnanediol glucuronate in urine, which contains the isotope [8]. The close relationship between progesterone and adrenal steroids can be seen in its in vitro transformation into deoxycorticosterone, through hydroxylation.

Pregnanediol is an inactive physiological reduction derivative of progesterone, isolated by Marrian in 1929, from pregnant women's urine Its chemical relationship with progesterone results clearly from the above-mentioned formulae. It can easily transform itself into progesterone in the laboratory. Blood and urine include only small values of progesterone. Similar to estriol and testosterone derivatives, pregnanediol is excreted in conjugated form with the glucuronic acid [9]. The liver is the main centre for the transformation of progesterone into pregnanediol, and also for the conjugation with glucuronic acid.

There are numerous confusions between progesterone and progestin (chemical progesterone received from medical supplements). The progesterone *produced* by the human body has a unique molecular structure; being the *natural* progesterone hormone:

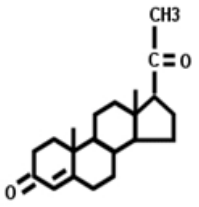

Fig. 3. Natural progesterone

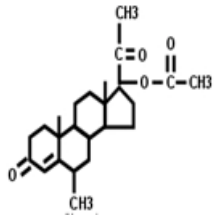

Fig. 4. Progesterone with modified molecular structure

Progesterone and progestatives include a series of drug substances with the following effects:

-*luteomimetics,* determining the secretory modification of the endometrium, preparing it for implantation;

-*progestagena,* favoring the development of the placenta and inhibiting the motility of the uterus;

-*stimulating the proliferation of mammal tissue*, acting only after previous preparation through its oestrogens.

Unlike the *natural progesterone* that can be administered in various ways: parenteral, oral, transcutaneous, vaginal, as gel or soft capsules, the *synthetic progestogens* derivatives can only be administered per os, acting for a longer period of time. Presently, there are the following types of progestins:

-progestogens, pregnan or C-21 compounds derivatives, including the 17-OH derivatives of progesterone;

-19-norprogesterone or norpregnan derivatives;

-19-nortestosterone or norsteroid derivatives.

Progesterone-a key role in every stage of human pregnancy

Progesterone is a key steroid hormone in the development of early pregnancy, helps keeping the myometrial state of calm in the second part of the pregnancy and is involved in setting off both term and preterm labour, when the functional diminution of progesterone at uterus level is noticed, without the modification of the plasmatic level [10].

In *early pregnancy*, the progesterone produced by the luteal corpus is indispensable for maintaining the pregnancy until the placenta takes over this function at weeks 7-9 of gestation. Thus, it is named *pro-gestational steroid hormone,* PR antagonists inducing abortion if administered before the first 7 weeks (49 days) of gestation.[11]

During pregnancy, a progressive growth of progesterone level is recorded between weeks 9 and 32, often 100 times higher than the value recorded before pregnancy. The progesterone concentration is higher in case of *multiple pregnancy* compared to *single pregnancy* [12].

The role of progesterone in late pregnancy is acknowledged but it is less clear. Thus, its main mode of action at the level of the myometrium is unknown.

Progesterone and progestins have a direct and indirect miorelaxing effect depending of the concentration [12] assuming they limit the production of stimulating

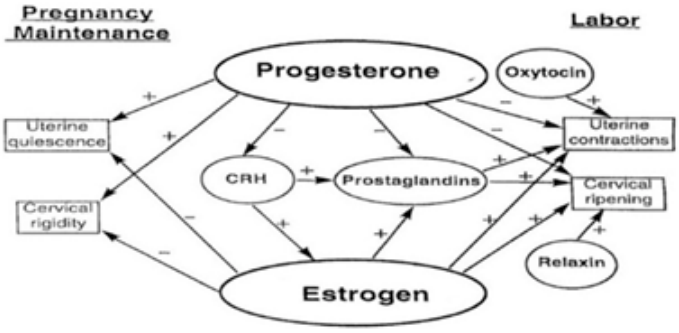

Fig. 5. The steroid hormones action of mode [14]

prostaglandins and inhibit the myometrial contractile mechanisms (ionic channels, oxytocin, prostaglandin receptors and gap junctions) [13].

Progesterone is necessary for the success of a pregnancy and the reduction of its seric concentration lays at the base of the beginning of labour. In 1960, Csapo and Pinto-Dandas stated the hypothesis of *progesterone blockage*, that suggests that myometrial *calmness* of human pregnancy is maintained by the high level of progesterone [15]. Although the systemic reduction of progesterone level may not be directly correlated to the beginning of labour in humans, it is obvious progesterone exerts its influence indirectly by the functional decrease of uterine levels. The level of the circulating progesterone remain high until after the expulsion of the placenta suggesting that the decrease of progesterone level is not compulsory / indispensable at term.

The molecular mechanism of progesterone action

The literature approaching this subject suggested six molecular mechanisms:

The functional decrease of progesterone prior to delivery can be mediated by modifications on myometrial progesterone receptors [16] and the latest studies indicated modifications of the receptors from the cervix [17] and the membrane [18]; 18the expression of A and B (PR-A and PR-B) progesterone receptors has changed along with the increase of PR-A/PR-B relationship.

There is only one gene for PR located on chromosome 11q22-q23 that uses separate promoters and produces 2 distinct isoforms: PR-A (94kD) and PR-B (116KD) that is identic to the first except for the 165 extra amino acids [19].

The beginning of labor at term is associated to the increase of the myometrial relationship PR -A/ PR -B resulting a decrease of progesterone action.[20] During almost the entire pregnancy period, progesterone seems to be reducing the myometrial response to oestrogen by inhibiting the alpha myometrial estrogenic receptors (ER á); this explains why the myometrium is refractory to the high levels of circulating oestrogens from gestation. At term, the decreasing functional progesterone modifies the suppression of myometrial ER α that makes the myometrium respond better to oestrogens, which turn it into a contractile phenotype [4].

The relationship between the decrease of functional progesterone and the activation of functional oestrogen can be the key decisive mechanism for the endocrine / paracrine control of term labour. This model explains why the interruption of the action of progesterone can alone be the *trigger* of delivery [4].

Progesterone is an anti-inflammatory agent

Inflammation plays a well-established role in initiating and maintaining term or premature labour. The key of inflammation mediators includes cytokines (IL-1β and IL-8) and prostaglandins. The production of prostaglandins is regulated by a number of cytokines via 2-cyclooxygenase enzyme (COX-2) from amnion, decidua-chorion and myometrium. An important regulator of these relationships seems to be the kB nuclear factor (NF-kB).

The endogen levels of prostaglandins in the decidua are lower during pregnancy, but their levels increase in labouring women. They are also used to induce pharmacological labour or abortion [21].

Progesterone receptor coactivators in the myometrium decrease when approaching the term

Using the semi-quantitative and real time PCR methods together with immunohistochemistry, Condon et al. indicated that the values of progesterone receptor coactivators as well as other transporting proteins are low in the myometrium during labour compared to the pregnancy period. The decrease of progesterone also makes the myometrium be more sensitive to contractile stimuli. Thus, it is partly explained why we can encounter a decrease of functional progesterone in the uterus without significantly modifying the value of circulating progesterone [22].

Progesterone can interfere with placenta regulated cortisone

More and more data suggest that placental CRH (corticotrophin releasing hormone) can act a key role during labour. The CRH levels in maternal plasma are undetectable at routine controls at non-pregnant women. They increase 3 weeks before term and spontaneous preterm labour. Cortisol and progesterone seem to have antagonic effects in the feto-placentary unit. The interesting element is that the two types of progesterone receptors can modulate in a different manner the genetic expression of placental CRH. Thus, PR-A decreases the activity of CRH, and PR-B has the opposite effect [20]. Most probably, the dominant environment of cortisol in the feto-placentary unit exactly before the beginning of labour can be influenced on various autocrine/paracrine ways to overcome the efforts of progesterone to maintain uterine calmness and prevent myometrial contractions.

Progesterone can also act on non-genomic ways

In 1998, Grazzini et al. demonstrated clearly that progesterone metabolites, not progesterone itself, are capable of entering the cell, coupling and distorting the receptor's conformation to oxytocin, preventing this way the coupling with oxytocin and inhibiting contractions [23].

Possible role of membrane PR from the myometrium

The existence of a distinct membrane connected to the PR is well known in animals and recently it has also been confirmed in humans but the phenomena are still under discussion and are far from being solved [24].

Studies that experimented the role of progesterone in pregnancy

Due to its numerous roles in initiating and maintaining the pregnancy, progesterone was the natural choice for the treatment and prevention of premature birth [25].

Premature birth remains an issue of interest since it is the main cause of morbidity and perinatal and infantile mortality, even though at global level the survival rate and the health state of premature children has recorded a progress. NP aetiology is often named *multifactorial*. An efficient screening test of the PTB and must be part of the obstetrical practice. The premature birth risk is in inverse proportion to the length of the cervix. When performed with adequate technique, it has been shown a high objectivity and reduced inter- and intra-operative variability. The screening method is easy and in the majority of cases can be associated to the mid-trimester ultrasound screening. A big advantage of the ultrasound screening is that it can easily identify the pregnant women with increased risk of PTB, but who do not require treatment. In fact, over 60% of the high-risk pregnant women, such as

those with history of PTB, have a CL at least 25-30 mm up to 24 weeks and do not require any intervention. Thus, the useless and expensive treatments are reduced, such as: bed rest, the transfer of the pregnant woman, administration of corticosteroids and tocolytics [26]

Various studies have experimented the use of progesterone in different combination for NP prophylaxis. The earliest trial was in 1975 and it was conducted on 43 patients who received progesterone i.m. or placebo every week [27]. The authors identified a protective effect of progesterone, which significantly increased the duration of the pregnancy, increased the foetal weight at birth and decreased the perinatal mortality rate.

Csapo (1981) was a champion in this field and he was the first to state the hypothesis that progesterone inhibits uterine contractility However, Csapo did not consider the cervix to be important in setting off and maintaining labour and delivery [15].

Professor Marc Keirse conducted a meta-analysis of the studies published on the prophylactic action of progesterone in premature birth, more precisely, 7 trials that indicated the decrease of premature cases in high risk patients [28].

In 2003 there were reported two multicentre trials. Meis et al. recruited 463 patients with NP antecedents and some of them were administered intramuscular progesterone [29]. Fonseca et al. reported a randomized trial on 142 pregnant women to whom he administered intravaginal progesterone versus placebo, between 24-34 gestational weeks. Both conducted to a significant reduction of the number of premature births, demonstrating at the same time the reduction of uterine contractility through tocometrical recordings for 60 s weekly (once a week) [30].

Further studies were also conducted on rats: in 2010 and 2011. Kuon et al. [31] administered subcutaneous and intradermic progesterone, noticing that it completely blocks delivery. Garfield et al. indicated the decrease of electric myometrial activity using the electromiographic recording at rats, at different gestational moments and before normal birth, at term, after the administration of progesterone [32].

Conclusions

The role of steroid hormones on pregnancy and labour was long discussed and subjected to controversies. Progesterone demonstrated its multiple benefits in initiating and maintaining a pregnancy and it was the natural choice in preventing preterm delivery.

Both new-born babies and their families are affected by the experience of premature birth emotionally, physically, financially and, unfortunately, prematurity is the main cause of death in the first month of life.

Despite the fact that the circulating progesterone levels during labour do not differ from the levels recorded a week before, it is obvious that labour, whether premature or at term, is preceded by the *functional* decrease of the active progesterone level on the uterus.

These data are clinically translated into the necessity of using additional progesterone in the pregnancy cases with high risks of premature birth, fact approved and recommended by ACOG (American College of Obstetricians and Gynecologists) since 2003. Nonetheless, the benefit of progesterone in case of premature labour has not been proven yet. Still, there are studies that indicated a synergic effect of progesterone with the tocolytics regularly used on in vitro myometrial contractility.

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