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Pharmacokinetics and Pharmacodynamics of Progestogens Used in Assisted Reproduction

Abstract

Progesterone is secreted from the corpus luteum in a pulsatile manner. During the mid to late luteal phase, the plasma concentrations may rapidly fluctuate up to eight-fold within a few hours. In vitro fertilization (IVF) treatment can promote the occurrence of luteal phase insufficiency due to ovarian hyperstimulation combined with pituitary down-regulation. Therefore, progesterone or dydrogesterone (DYD) is routinely administered in IVF treatment in order to improve embryo implantation during the luteal phase. In contrast to DYD, which is always orally used, progesterone is predominantly administered parenterally (vaginally, sc or im) in assisted reproductive technology cycles. Oral administration of progesterone causes extensive metabolism resulting in the formation of more than 30 metabolites, some

of which may cause anesthetic/sedative effects. The use of a direct radioimmunoassay (RIA) without prior separation of progesterone from its metabolites may simulate falsely high serum concentrations of progesterone owing to cross-reaction of some metabolites with the specific antiserum of the RIA. This effect is not observed after parenteral treatment with progesterone due to minimal metabolism. The rigid retro structure of DYD allows only the formation of a few metabolites. After vaginal administration of progesterone, the endometrial and myometrial progesterone concentrations are markedly higher than those after the injection of an oily suspension of progesterone. This suggests a direct diffusion and deposition of progesterone in the uterus (uterine first-pass effect). The systemic absorption of transdermal progesterone is low.

Keywords

Progesterone · Dydrogesterone · Progestins/drug administration routes · Progesterone metabolites · In vitro fertilization

[Introduction]

In in-vitro fertilization (IVF) treatment, the support of the luteal phase through treatment with progesterone or dydrogesterone plays an important role, because the previous hyperstimulation of the ovaries in combination with the pituitary down-regulation can cause a luteal phase defect, which with a deficient release of progesterone is associated [1].

» Progesterone is secreted in a pulsatile manner

Since a low progesterone level can impair the implantation process, the pregnancy rate can be supported by a suitable substitution with progesterone. However, the detection of a progesterone deficiency by means of a one-off determination of the progesterone concentration in the serum is problematic because progesterone, like luteinizing hormone, is secreted in a pulsatile manner. The progesterone level can fluctuate up to eight times within a few hours, especially in the middle and late luteal phase [2].

Meaning of progesterone and dydrogesterone

Progestogens are defined as steroid hormones which cause the secretory transformation of an endometrium that has proliferated under the influence of estrogen and which maintain pregnancy. The latter only applies to progesterone and synthetic dydrogesterone in women. Progesterone is formed in the corpus luteum during the postovulatory phase and in the placenta during pregnancy. In the luteal phase, progesterone reaches a serum concentration of 25 ng/mL, which can rise to 200 ng/mL during pregnancy. In general, oral progesterone treatment leads to the secretory transformation of a proliferative endometrium in a dose-dependent manner and prevents endometrial hyperplasia.

» In contrast to progesterone, dydrogesterone has no central effects

Dydrogesterone (9β,10α-pregna-4,6-diene-3,20-dione) is a retroprogesterone whose steric configuration differs significantly from that of progesterone (Fig. 1). In contrast to progesterone, dydrogesterone has no central effects because, due to its retro structure, it cannot be reduced to the pregnanolones. Therefore it has no thermogenic effect, has no sedative effect, and neither inhibits gonadotropin release nor ovulation [3].

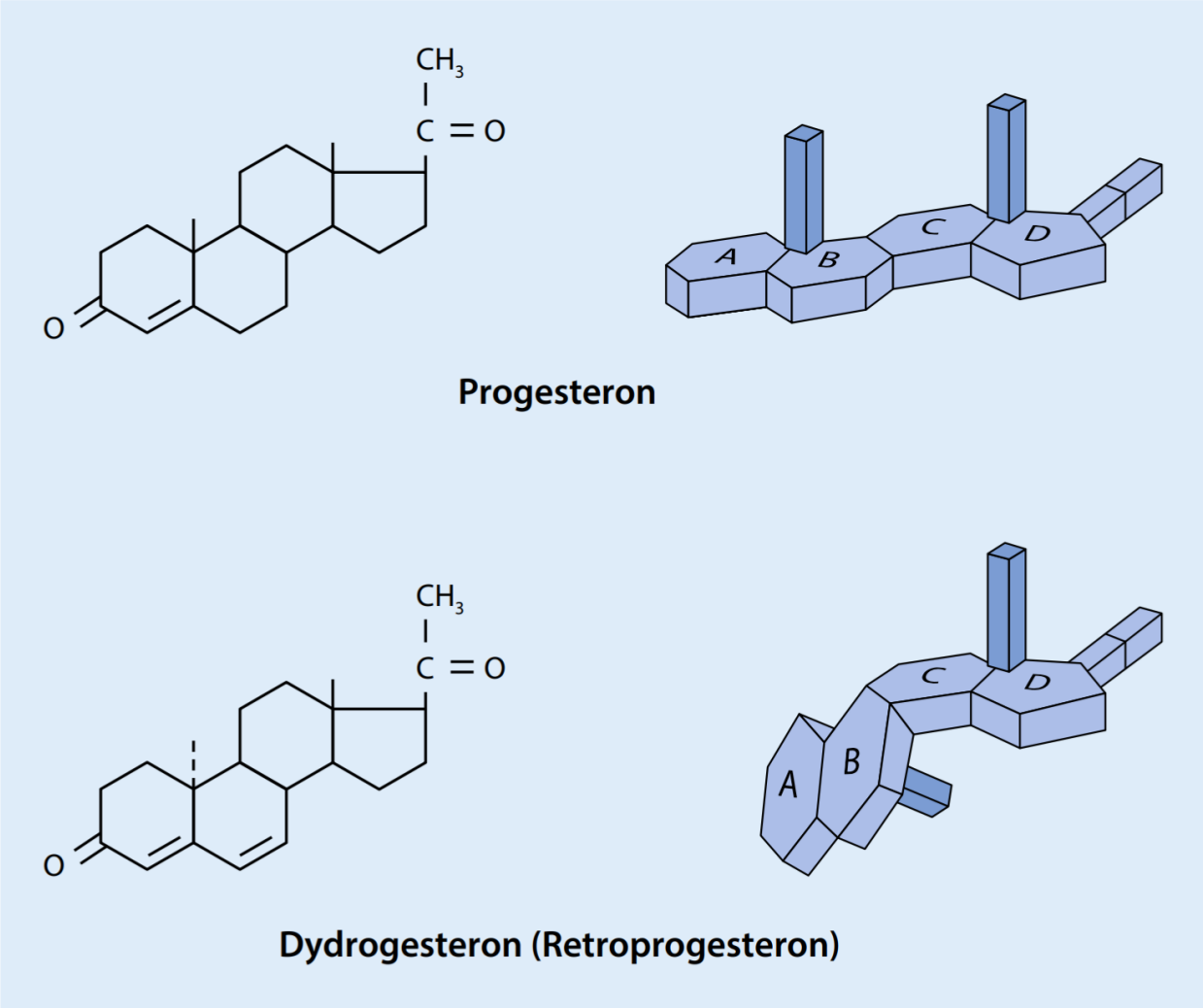

Fig. 1. Structural formulas and steric configuration of progesterone and dydrogesterone

Mechanisms of action of progesterone

The effects of the progestogens are based on genomic interactions with the two progesterone receptors (PR) PR-A and PR-B as well as on rapid non-genomic processes in the cell membranes or cells. Since the PR is induced by the estrogens, the progesterone effect in the endometrium is estrogen-dependent. The activated receptor PR-A not only causes suppression of PR-B, but also that of the estrogen (ER), androgen (AR), glucocorticoid (GR), and mineralocorticoid receptor (MR; [3]).

> » Increased synthesis of PR takes place in the proliferation and early secretory phase

An antiestrogenic effect of the progestogens is also based on the activation of the 17β-hydroxysteroid dehydrogenase type 2, which converts estradiol into the weakly active estrone. Among other things, this inhibits the estrogen-dependent mitotic activity in the endometrium and prevents the development of endometrial hyperplasia.

During the entire proliferative phase of the endometrium, the ER is strongly expressed in the nuclei of the epithelial and stromal cells, but drops dramatically in the middle and late phases. There is a transition from strong expression of ER in the early secretory phase to weak expression in the functionalis of the middle and late secretory endometrium [4].

The PR is found in the nuclei of the epithelial and stromal cells as well as the myometrial smooth muscle cells, with the receptor content varying in the menstrual cycle. An increased synthesis of the PR takes place in the proliferation phase and in the early secretory phase, but on postovulatory day 4 there is a sharp decrease to a low level during the middle and late secretory phase. The PR is even moderately to strongly expressed in the stromal cells during the secretion phase, but not in the vascular smooth muscle cells or endothelial cells [5].

Effects and partial effects of progesterone

Progesterone has an antihypertensive and antimineralocorticoid effect, which causes a compensatory increase in the aldosterone level of 70% in the luteal phase [6]. Progesterone binds to the MR with a higher affinity than aldosterone and competitively inhibits the effect of aldosterone and 11-deoxycorticosterone (DOC) on the MR. However, progesterone can be converted into the potent mineralocorticoid DOC.

In contrast, the partial effect of progesterone as a glucocorticoid is less pronounced [7]. The slight antiandrogenic effect of progesterone, for example in the skin, does not come about through an interaction with the AR, but rather through a competitive inhibition of 5α-reductase. This reduces the reduction of testosterone to the much more potent dihydrotestosterone (DHT).

In the luteal phase, the rising progesterone level increases the body temperature by 0.4–0.6 °C, relaxes the smooth muscles, has an anti-inflammatory effect, and influences immune response, pancreatic function, and insulin release [8]. Oral treatment with 100–300 mg progesterone lowers blood pressure in a dose-dependent manner and reduces vasomotor symptoms [8, 9]. In single doses of 300 to 1200 mg, progesterone impairs information processing and memory function [10]. Finally, as an intermediate product, progesterone plays an important role in ovarian and adrenal steroid biosynthesis, is involved in the regulation of gonadotropins, and influences the psyche.

Progesterone metabolism

In the pharmacokinetics of progesterone, the binding to transport proteins is important because it influences the proportion of the free hormone and thus its biological activity. Progesterone is 0.6% bound to sex hormone binding globulin (SHBG), 17.7% to corticosterone binding globulin (CBG), and 79.3%—but with low affinity—to albumin. The binding of steroids to proteins, for example the interaction with hormone receptors, is dependent on the association and dissociation constants and, above all, on the local concentrations in the cells, in accordance with the law of mass action.

» Progesterone metabolites can produce anesthetic or sedative effects

About 60–65% of the metabolism of progesterone takes place via the 5α reduction of the keto groups and the Δ4 double bond, the number and amount of the metabolites depending on the method of application. Basically, with parenteral application of progesterone (including vaginal, intramuscular, or subcutaneous), higher serum progesterone levels are achieved than with oral application due to the low metabolism. The

progesterone level after using a vaginal gel with 90 mg progesterone is about 4 to 5 times higher than after oral administration of 100 mg progesterone (Table 1; [11]). This difference is due to the strong inactivation of the orally administered progesterone in the intestinal tract and in the liver. Accordingly, even after intramuscular treatment with 100 mg progesterone, the serum level of progesterone is significantly higher than after oral administration of the same dose [12]. Conversely, after oral progesterone treatment, high serum concentrations of many progesterone metabolites arise due to the high level of metabolism. After oral progesterone treatment the concentrations of DOC are about 7 times and those of 5α-pregnanolone 3.5 times higher than after vaginal application (Table 2; [13]). The excretion of the metabolites, in particular that of the end product pregnanediol, occurs mainly after conjugation to water-soluble glucuronides or sulfates.

Table 1. Progesterone pharmacokinetics after a single vaginal or oral treatment with progesterone. (Modified from Levine and Watson [11])

	Gel vaginal		**Oral capsule**	
	90 mg progesterone (Crinone® 8%, Merck, Darmstadt)		**100 mg Progesterone (Prometrium, Dr. KADE/BESINS Pharma GmbH, Berlin)**	
Method	LC-MS (MW ± SD)	RIA	LC-MS (MW ± SD)	*RIA* (MW ± SD)
C_{max} (ng/mL)	10.5 ± 0.5	10,5	2.4 ± 5.0	*19.4 ± 12.6*
t_{max} (h)	7.7 ± 3.7	7,7	1.0 ± 0.4	*1.0 ± 0.4*
$AUC_{0–24\ h}$ (h·ng/mL)	133.3 ± 14.6	–	3.5 ± 5.2	–

After oral use of progesterone, the RIA measures incorrectly high progesterone levels, as some progesterone metabolites that are formed in the body after oral use are also measured by cross-reactions (highlighted in italics)

AUC "area under the concentration curve" (0–24 h), C_{max} maximum serum concentration (determination of the progesterone concentration with LC-MS or RIA), *LC-MS* liquid chromatography-mass spectrometry, *MW ± SD* mean values ± standard deviation, RIA radioimmunoassay, t_{max} Time to maximum

Table 2. Plasma concentrations of progesterone and its metabolites after oral and vaginal treatment with 100 mg of micronized progesterone. (Modified from Nahoul et al. [13])

	Oral progesterone		**Progesterone vaginal**		**Oral progesterone**		**Progesterone vaginal**	
Time	**Prog**	**DOC**	**Prog**	**DOC**	**5α-Preg**	**5β-Preg**	**5α-Preg**	**5β-Preg**
h	**ng/mL**	**pg/mL**	**ng/mL**	**pg/mL**	**ng/mL**	**ng/mL**	**ng/mL**	**ng/mL**
0	0.13	116	0.19	33	0.55	0.12	0.11	<0.05
2	1.50	676	2.00	78	14.00	0.16	3.55	<0.05
4	0.70	192	3.80	98	5.38	0.31	1.20	<0.05

6	0.36	112	4.70	67	3.07	0.48	0.76	<0.05
24	0.21	124	4.50	45	0.89	0.55	0.17	<0.05
Prog Progesterone, *DOC* 11-Deoxycorticosterone, *5α-Preg* 5α-Pregnanolone, *5β-Preg* 5β-Pregnanolone								

There are more than 30 different progesterone metabolites that have different hormonal partial or central effects (Table 3; [14]). After oral administration of progesterone, the serum levels of 5α- and 5β-pregnanolone and of pregnane-3α,20β-diol and 5β-pregnan-3α-ol-11,20-dione in particular increase. This is important because it is linked to the γ-aminobutyric acid subtype A ($GABA_A$) receptor and produces anesthetic or sedative effects. Of 8 women who had taken high doses of progesterone and were more or less sedated, one woman was in deep sleep after taking 400 mg of micronized progesterone for a period of 2 hours. An analysis of the metabolites in the serum of this woman revealed some anesthetically effective steroids with sometimes very high concentrations: 5α-pregnan-3α-ol-20-one (130 ng/mL after 2 h), 5β-pregnan-3α-ol-20-one (80 ng/mL after 2 h), 5β-pregnan-3α,20α-diol, 5β-pregnan-3α-ol-11,20-dione (84 ng/mL after 6 h), and 5β-pregnan-3α,20β-diol (46 ng/mL after 6 h; [15]).

Table 3. Metabolites of progesterone and dydrogesterone and their partial effects. (Modified from Arafat et al. [15], Wiebe et al. [16], and Lanisnik Rizner et al. [19])

Steroid	**Partial effects**	**Short form**
Progesterone	Progestogen, androgen, antiandrogen, mineralocorticoid, antimineralocorticoid, glucocorticoid, antiglucocorticoid	–
3α-Dihydroprogesterone	Antimitotic	3α-DHP
20α-Dihydroprogesterone	Antimitotic, progestogen	20α-DHP
5α-Dihydroprogesterone	Mitotic	5α-DHP
5β-Dihydroprogesterone	–	5β-DHP
17α-Hydroxyprogesterone	Inactive	17-OHP
17α-Hydroxyprogesterone acetate	Progestin	17-OHPAc
4-Pregnen-3α,20α-diol	Antimitotic	–
5-Pregnen-3β-ol-20-one	–	–
5β-Pregnane-3α, 20α-diol	Anesthetic/sedative	–
5β-Pregnane-3,20-dione	Anesthetic/sedative	–
5α-Pregnan-3α-ol-20-one (allopregnanolone)	Anesthetic/sedative, mitotic	–
5α-Pregnan-20α-ol-3-one	Mitotic	–
5β-Pregnan-3α-ol-20-one (isopregnanolone, epipregnanolone)	Anesthetic/sedative	–
5β-Pregnan-3α-ol-11,20-dione	Anesthetic/sedative	–
5β-Pregnane-3α,20β-diol	Anesthetic/sedative	–

5α-Pregnane-3α,20β-diol (pregnanediol)	Inactive	PGD
5α-Pregnan-3α,20α-diol	Mitotic	–
11-Deoxycorticosterone (corticosterone)	Mineralocorticoid	DOC
Dydrogesterone	Progestin, no further clinically relevant partial effect	Dydro
20α-Dihydrodydrogesterone	Slightly progestogenic, no further clinically relevant partial effect	DHD
16α-Dihydrodydrogesterone	–	16-DHD
21-Dihydrodydrogesterone	–	21-DHD

Further metabolites are 20α-dihydroprogesterone (20α-DHP), which has 25–50% of the progestogenic effectiveness of progesterone, and the potent mineralocorticoid DOC, while 17α-hydroxyprogesterone (17-OHP) has no hormonal activity. The inactive end product of the various metabolites is pregnanediol, which is mainly excreted as a conjugate. The mitotic activity of 5α-dihydroprogesterone (5α-DHP) and the antimitotic effect of 3α-dihydroprogesterone (3α-DHP) and 20α-DHP could be of particular clinical importance, including with regard to the risk of breast cancer (Fig. 2; [16]). The important progesterone metabolites also include 5β-DHP, pregnenolone, 5α-pregnanolone, and 3β- and 5β-pregnanediol (Table 3).

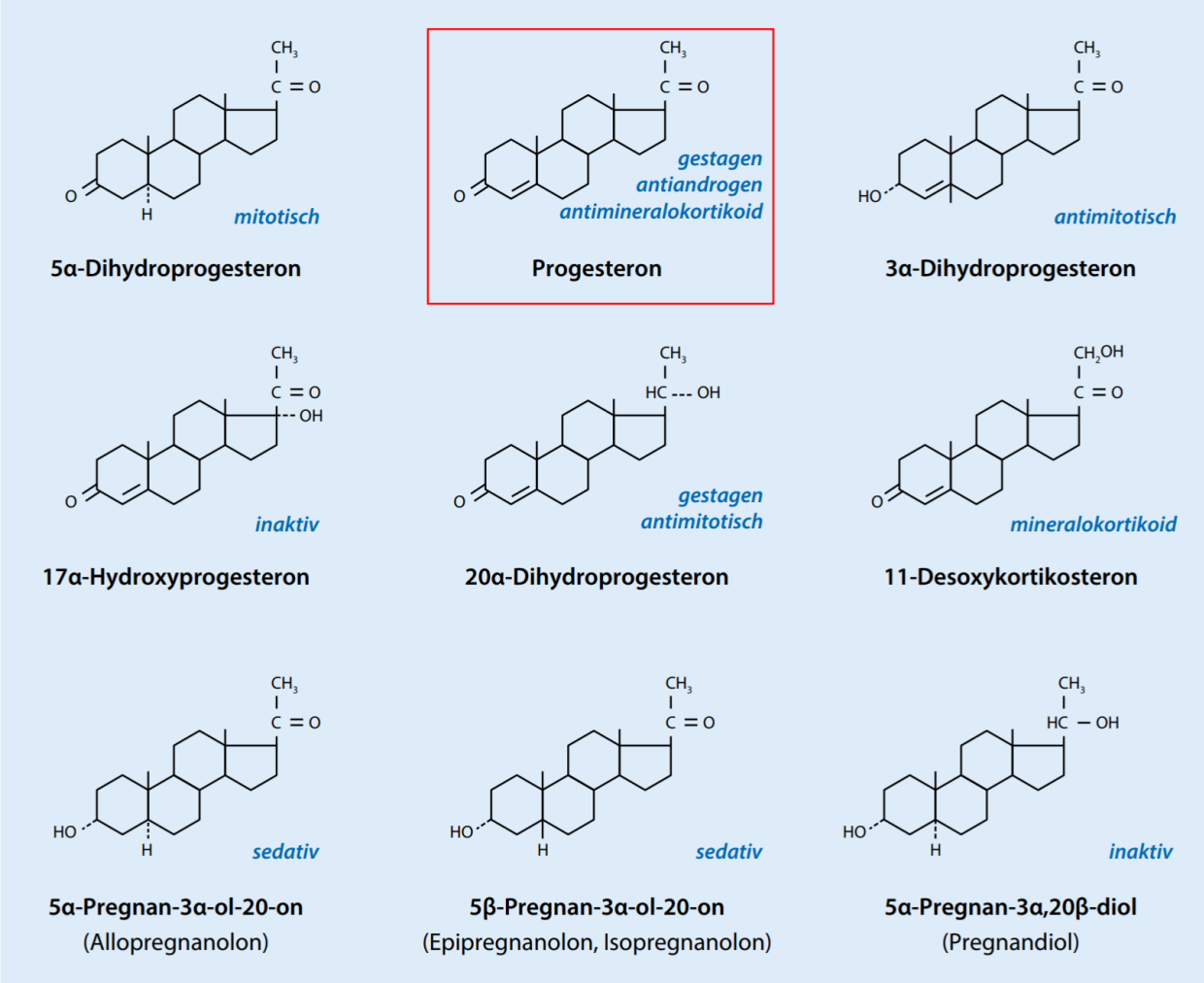

Fig. 2. Progesterone metabolites with special hormonal, central, or mitogenic properties

While the reduction of progesterone to 5β-pregnanolone takes place largely in the liver, the 5α reduction takes place predominantly in the small intestine. High DOC concentrations that occur after oral progesterone administration also indicate intestinal conversion, as there is no steroid 21-hydroxylase in the liver [13]. High DOC concentrations that occur after an intramuscular injection of 100 mg progesterone are due to a conversion of progesterone in the peripheral tissue [12].

Potency of progesterone and dydrogesterone

The oral dosages of progesterone and dydrogesterone recommended for endometrial protection correspond to their potency, which can be determined experimentally. In a study with postmenopausal women on estrogen substitution, various biochemical parameters of the endometrium were analyzed. Compared to a potency of 100% for norethisterone (NET), the potency indicated for progesterone was 0.2%, for dydrogesterone 2%, and for medroxyprogesterone acetate (MPA) 10%. Accordingly, the following dosages of these orally administered progestogens had an approximately equally strong effect on an endometrium pretreated with estrogens: 0.35 mg NET, 5 mg MPA, 10 mg dydrogesterone, and 200 mg progesterone [17]. 17-OHP has no progestogenic activity, but the esterification of the 17α-hydroxy group with carboxylic acids gives rise to derivatives with a pronounced progestogenic effect, for example

17-OHP acetate. 17-OHP caproate, which is no longer approved in Germany, also has a strong depot effect.

Effects and partial effects of dydrogesterone

Oral sequence therapy with 1 mg estradiol and 5–10 mg dydrogesterone or with 2 mg estradiol and 10–20 mg dydrogesterone causes an atrophic or secretory endometrium in most postmenopausal women and prevents endometrial hyperplasia [18]. It is known from clinical use that dydrogesterone has no relevant androgen, estrogen, glucocorticoid, or mineralocorticoid activities. The interactions of dydrogesterone, its main metabolite 20α-dihydrodydrogesterone (DHD), and progesterone with various steroid receptors and their genomic and non-genomic effects were investigated in an in vitro study [19].

The binding of a steroid hormone to a specific receptor can either trigger an agonistic reaction by activation or prevent the effect by blocking the receptor as an antagonist. The effectiveness of the hormone depends on the binding affinity and the steroid concentration. Dydrogesterone and DHD show a stronger affinity for PR than for GR, AR, and ER, which is probably due to their rigid configuration, which does not allow binding to the other steroid receptors ([19]; Fig. 1). Although dydrogesterone has only about 20% of the binding affinity of progesterone on the PR, it has a stronger progestogenic effect due to its pharmacokinetic properties. DHD has the same affinity for PR as dydrogesterone, but less for AR and GR. In contrast, dydrogesterone, DHD, and progesterone do not bind to ERα and ERβ.

The non-genomic effects include the inhibition of androgen synthesis enzymes, including 17β-hydroxysteroid dehydrogenase and 5α-reductase. The antiandrogenic effect of progesterone is based primarily on the pronounced inhibition of 5α-reductase type 2, while dydrogesterone and DHD do not inhibit this enzyme. Progesterone, dydrogesterone, and DHD reduce the biosynthesis of testosterone in vitro by inhibiting 17α-hydroxysteroid dehydrogenase types 3 and 5, but the effect seems to be of no clinical significance due to the low plasma concentrations of these steroids after oral administration.

Analyses show that the progestogenic effect of progesterone and dydrogesterone is comparable, but that of DHD is significantly lower. With regard to its androgen, antiandrogen, glucocorticoid, and mineralocorticoid properties, however, progesterone differs from dydrogesterone and DHD [19]. In contrast to its metabolites, progesterone has a similarly strong antimineralocorticoid effect as spironolactone, while the antimineralocorticoid effects of dydrogesterone and DHD are negligible [19].

Metabolism of dydrogesterone

The oral bioavailability of dydrogesterone is 28%, considerably higher than that of progesterone, which is <5%. After ingestion of dydrogesterone, the concentration of the main metabolite DHD is about 25 times higher than that of dydrogesterone [3]. In addition, the progestogenic effectiveness of dydrogesterone when administered orally is enhanced by the progestogenic effect of DHD. For the complete secretory transformation of a proliferative endometrium, 10–20 mg dydrogesterone per day is sufficient [6].

Due to the 9β,10α retro structure of dydrogesterone, the two double bonds cannot be reduced. The most important metabolic reactions are the reduction of the 20-keto group to DHD and the hydroxylation at C16α and C21 to 16α-OH-dydrogesterone and 21-OH-dydrogesterone (Table 3; [19]). The half-lives $t_{1/2\alpha}$ and $t_{1/2\beta}$ of dydrogesterone after oral administration are 5–7 h and 24 h, respectively, with 85% of the dose being eliminated after 24 h.

Progesterone pharmacokinetics

Problems with the determination of progesterone in serum

Because of the large number of progesterone metabolites that circulate in variable concentrations after oral administration, false-high progesterone levels must be expected when using an immunoassay. The cause is the cross-reactions of some progesterone metabolites, such as 5α- and 5β-DHP and 5α-pregnan-3β-ol-20-one [13]. For this reason, a radioimmunoassay (RIA) can only be used, for example, if the progesterone has previously been isolated by means of a suitable chromatographic separation. The determination of progesterone with exact liquid or gas chromatography-mass spectrometry (LC-MS or GC-MS; [14]; Table 1) is more reliable, albeit more complex.

The problem of cross-reactions does not play an essential role in parenteral progesterone treatment due to the low metabolism of progesterone. The specificity of the antiserum used is of particular importance for the comparability of the results of different RIAs, as it influences the cross-reactivity of the numerous progesterone metabolites [11].

The determination of the progesterone level, which was measured after oral administration of a capsule containing 100 mg of micronized progesterone, resulted in a false high peak value of 19.4 ng/mL in the RIA, which was 8 times as high as that with the exact LC-MS method which determined a true value of 2.4 ng/mL (Table 1). The cause was metabolites, the high concentrations of which simulated a false high concentration via a cross-reaction with the RIA antiserum [11]. In contrast, after intravaginal application of a gel containing 90 mg of progesterone, the metabolism of progesterone and thus the influence of cross-reactions is so low that the progesterone concentration of 10.5 ng/mL measured with the RIA agrees with the result of the exact LC-MS method (Table 1; [11]).

"Enzyme-linked immunosorbent assay"

As with the RIA, the “enzyme-linked immunosorbent assay” (ELISA) is a procedure based on an immune reaction between an antigen and a specific antibody. For this purpose, a specific antibody is produced in a separate standard process for the antigen, for example progesterone, which binds the antigen with high affinity. An enzyme is coupled to this specific antibody that cleaves an added chromogen, producing a dye, the intensity of which is measured in a photometer. The measured intensity of the color correlates with the concentration of the antigen (for example progesterone).

With regard to the cross-reaction of some progesterone metabolites, which leads to false high values of the progesterone concentration, the same restriction applies to the ELISA as to the direct RIA (Table 1).

Notice.

If a progesterone determination is carried out in the serum after oral progesterone application in the RIA or in the ELISA without prior chromatographic separation, one measures incorrectly high progesterone values, since progesterone metabolites are also measured! This problem does not exist with parenteral application (intravaginal, intramuscular, or subcutaneous).

Problems with the concentration of progesterone in saliva

The concentration of progesterone, which after transdermal application in the thin skin area (inner arm, thigh, stomach) quickly diffuses into the capillary blood and the salivary glands, is influenced by enzymes and binding proteins as well as the flow of saliva. The assumption that lipophilic progesterone is absorbed by erythrocytes and transported in the circulation does not apply. When women were treated with a progesterone cream, progesterone concentrations of <0.27 ng/mL were found in the erythrocytes, 1.1 ng/mL in plasma, and 25.8 ng/mL in saliva [20].

After 14 days of transdermal application of 80 mg of progesterone in the form of gel or cream, the progesterone levels in saliva and capillary blood were 10 times or 100 times higher than the serum concentrations [21]. However, because of the strongly fluctuating progesterone concentrations, progesterone determinations in saliva are not suitable for monitoring progesterone therapy [22].

Oral application of progesterone

After oral administration, progesterone is rapidly metabolized in the intestinal tract and during the first passage through the liver by reductases, dehydrogenases and cytochrome P_{450} enzymes, so that the bioavailability is <5% and the half-lives are only 6 min ($t_{1/2\alpha}$) and 42 min ($t_{1/2\beta}$). The low bioavailability can be increased by micronization and suspension of progesterone in oil or by application in a gelatin capsule.

Oral treatment with 100 mg of micronized progesterone is part of a rapid perception of the serum progesterone level to 1.5 ng/mL and the serum concentrations of 5α- or 5β-pregnanolone to 14 ng/mL and 3.6 ng/mL, the DOC level rose annually from 120 pg/mL to 680 pg/mL [13]. In women pretreated with estradiol, taking 200 mg micronized progesterone for 12–14 days prevented endometrial hyperplasia [23]. After ingestion of 100 to 400 mg progesterone, some very high serum levels of some anesthetically or hypnotically active metabolites could be measured within 2 to 6 hours [15], for example of 5α-pregnan-3α-ol-20-one (up to 130 ng/mL), 5β-pregnan-3α-ol-20-one (up to 80 ng/mL), 5β-pregnan-3α,20β-diol (up to 46 ng/mL), and 5β-pregnan-3α-ol-11,20-dione (up to 84 ng/mL).

After separation of important progesterone metabolites by means of column chromatography, an increase in progesterone in the serum to a maximum of 4.7 ± 1.15 ng/mL was found in the RIA after oral administration of 200 mg micronized progesterone [14].

Notice.

In summary, it can be stated that oral administration of 100 to 200 mg progesterone leads to an increase in serum levels to around 3–5 ng/mL, provided the laboratory diagnostics are carried out correctly, i.e. after chromatographic separation.

Parenteral application of progesterone

To avoid excessive metabolism of the orally administered progesterone in the intestinal tract and during the first passage through the liver, various parenteral routes of administration are available. Since this bypasses the gastrointestinal and hepatic first-pass metabolism of progesterone, the intramuscular, subcutaneous, or vaginal application of progesterone leads to higher and more uniform progesterone concentrations than oral treatment.

Intramuscular application of progesterone

After intramuscular injection of an oily suspension of 100 mg progesterone, the progesterone level rose within 8 hours to a maximum of 40 to 80 ng/mL, i.e. to values that were 2 to 3 times higher than in the middle luteal phase. After that, the progesterone levels decreased continuously, but remained in the range of the luteal phase values for the following two days. 20α-DHP reached a serum level of 3 ng/mL and 17-OHP a value of 0.9 ng/mL [12].

> » Dose-dependent depot effect with a progesterone level increased for up to 48 hours after IM administration

Another study with 12 women showed a similar result. After injection of an oily suspension of 100 mg progesterone, there was a rapid rise in serum progesterone within 2 hours, which reached a maximum of 68 ng/mL after 8 hours and remained at a high level for at least 24 hours, with large interindividual differences of 7 to 26 ng/mL. The intramuscular administration of 10 mg, 25 mg, and 50 mg of progesterone in oily suspension showed similar pharmacokinetics with maximum serum concentrations of 7 ng/mL, 28 ng/mL, and 50 ng/mL, respectively. Depending on the dose applied, a depot effect was shown with a permanently increased progesterone level for up to 48 hours, which was, for example, 5 ng/mL after injection of 100 mg progesterone.24].

Intravaginal application of progesterone

The intravaginal application of progesterone leads, depending on the dosage and composition of the preparation, to a rapid rise in the progesterone level in the blood and in the endometrium (uterine first-pass effect).

After using a vaginal suppository (VS) with 100 mg micronized progesterone or a vaginal gel (VG) with 90 mg progesterone, the serum progesterone levels rose to 4.7 ng/mL and 10.5 ng/mL, respectively [11, 13]. After vaginal application of a VS with a mixture of 100 mg progesterone in cocoa butter, progesterone levels of 9.5 to 19 ng/mL were observed within 4 hours, while peaks of 15 to 52 ng/mL were observed after rectal use of the same preparation [24]. The application of a vaginal capsule with 200 mg progesterone led to a progesterone peak of 5.2 ng/mL after 12 h, while after vaginal application of a gel with 90 mg progesterone a maximum level of 6.1 ng/mL was reached after 10 h. However, the bioavailability of the progesterone from the vaginal capsule was 50% higher compared to the VG, while there was no difference in terms of side effects—fatigue and weakness. Due to residues of the gel in the applicator, the progesterone dose actually applied in this study was greatly reduced to 60 mg instead of 90 mg [25].

After vaginal application of a VS containing 400 mg of progesterone, the progesterone level rose to a maximum of 16 ± 17 ng/mL within 5 hours. In addition, the metabolites 5α-pregnanolone (1.2 ng/mL), 5β-pregnanolone (0.3 ng/mL), 5α-DHP (0.8 ng/mL), and DOC (0.3 ng/mL) were formed in much lower concentration than after oral administration of 100 mg micronized progesterone [26].

In women who received transdermal substitution with increasing estradiol doses of 100 to 400 µg, cyclical treatment with a VG with 45 mg, 90 mg, or 180 mg progesterone was carried out every 2 days from day 15 to 27. The progesterone levels rose within 7 h to 3.9 ng/mL, 6.3 ng/mL, and 7.5 ng/mL and fell to 0.9 ng/mL, 1.3 ng/mL, and after 30 h, respectively 1.45 ng/mL. Although the progesterone levels achieved were significantly lower than in the luteal phase, there was a complete secretory transformation of the endometrium in all cases. This effect confirms the thesis of a direct transport of vaginally applied progesterone into the uterus (uterine first-pass effect; [27]).

» Vaginal progesterone therapy with 800 mg/day leads to the secretory transformation of the endometrium

During treatment with increasing estradiol doses over 26 days, 20 women with primary ovarian insufficiency who wanted to donate eggs were given an additional vaginal capsule containing 200 mg micronized progesterone (800 mg/day) every 6 hours or an oily suspension twice a day for 11 days with 50 mg progesterone (100 mg/day) administered im. The serum progesterone increased to 16 ng/mL within 6 hours after intramuscular injection, but only to 6.6 ng/mL after vaginal application. After 6 days of intramuscular treatment, the steady state progesterone level of 70 ng/mL was far higher than the serum concentration of 12 ng/mL after vaginal progesterone administration. However, the progesterone concentration in the endometrium was 11, 5 ng/g protein after vaginal application is considerably higher than after intramuscular injection (1.4 ng/g protein)—another indication of the uterine first-pass effect. There were no differences between the two treatment groups and a control group with regard to the histological, ultrasound, ER, and PR analyses. The results show that vaginal progesterone therapy (800 mg/day) leads to a secretory transformation of the endometrium and is suitable for luteal phase support in the context of assisted reproduction [28].

In a randomized, double-blind study, postmenopausal women were treated continuously over 3 cycles with 0.625 mg conjugated estrogens as well as sequentially from day 17 to 27 on every other day with a VG with 45 mg or 90 mg progesterone. After 10-16 h, progesterone levels in the plasma of 4.6 ng/mL and 6.8 ng/mL were measured. Despite these relatively low progesterone levels, only one of the women treated with 45 mg progesterone showed a proliferative endometrium [29]. As the cause of this good endometrial effect of the low-dose VG, the uterine first-pass effect on the one hand and the bioadhesive properties of the gel on the other hand were discussed. It is assumed that prolonged contact of the larger area of the vaginal epithelium with the gel leads to greater absorption of the progesterone.

In 3 consecutive treatment cycles, women who had been pretreated with 2 mg estradiol twice a day also received a VS with 100 mg, 200 mg, or 400 mg progesterone (Cyclogest®, Gedeon Richter Pharma GmbH) twice a day from day 15 to 24, Cologne) or once a day a VG with 90 mg progesterone (Crinone®, Merck, Darmstadt) or a VS with 400 mg [30]. The pharmacokinetic studies were performed on the first (day 15) and last day of progesterone use (day 24, steady state). After the first application, the progesterone levels after application of the VS of 200 and 400 mg over 12 h were roughly comparable, while they were significantly lower after the application of 90 mg (VG) or 100 mg (VS). After repeated use, the levels of all preparations were higher than those after the first use. The highest concentrations were measured after application of VS twice a day with 400 mg of progesterone (Fig. 3, Table 4; [30]). They were more than twice as high as after using the VG once a day. Large inter-individual fluctuations were found in all treatment groups: the maximum progesterone levels after vaginal use of the gel were between 3.5 and 20.5 ng/mL and after twice daily use of the VS with 400 mg between 12 and 41 ng/mL. The endometrial biopsies on day 23 showed that the secretory transformation triggered by the VS with 200 mg or 400 mg applied twice daily was comparable to the effect of 90 mg VG. In contrast, the other doses (100 mg twice a day or 400 mg once a day) were less effective. At the time of the endometrial biopsy in the cycle, the late secretory stage of the endometrium, which is characteristic of adequate support of the luteal phase, observed more frequently with the VS with 400 mg progesterone applied twice a day than with the lower-dosed VS or the VG. In addition, the incidence of bleeding and spotting on the last days of treatment was lower with the VS applied twice a day (400 mg) than with the other preparations [30].

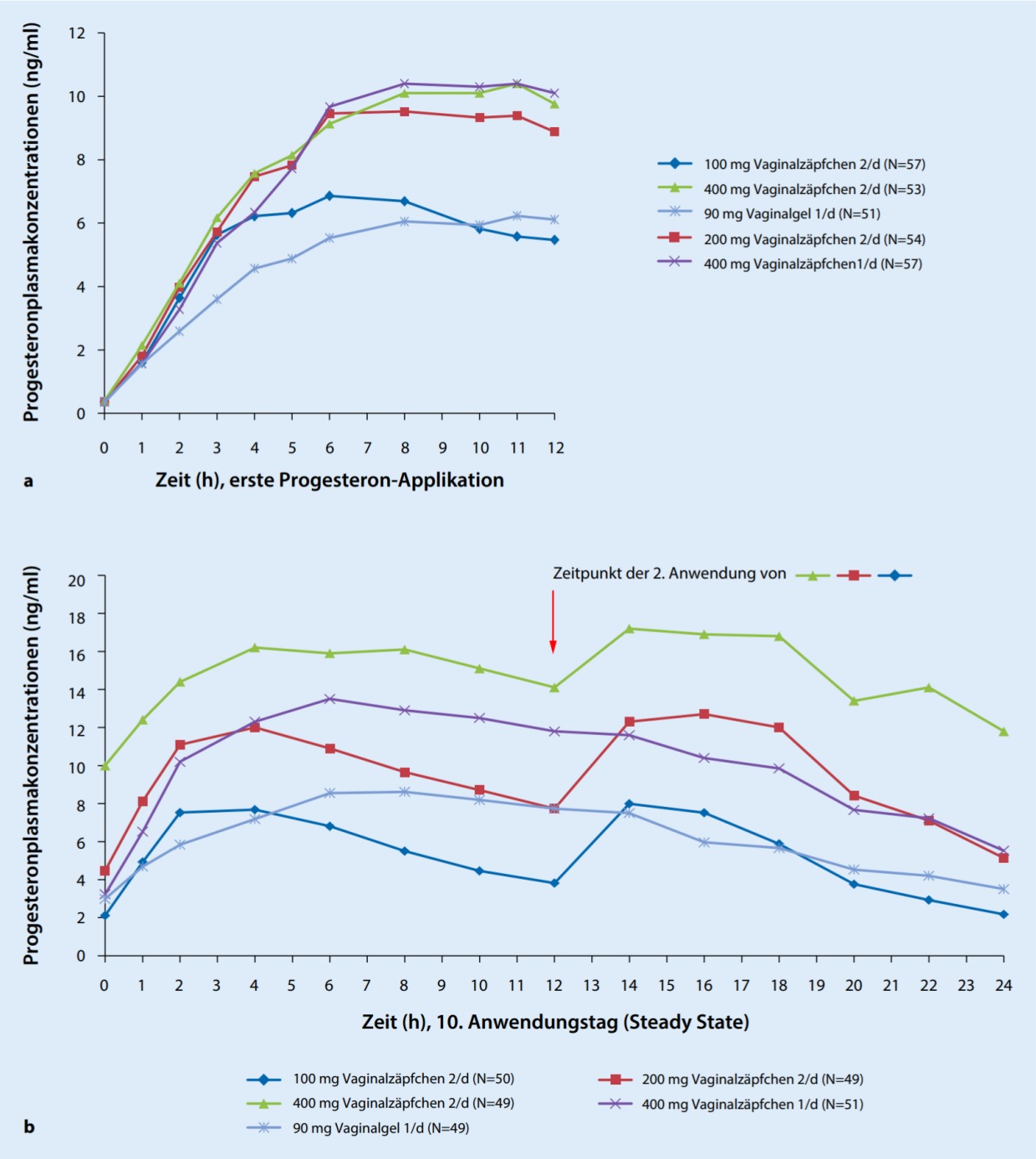

Fig. 3. Plasma level of progesterone after the first (a) or several days of repeated application (b) of various vaginally applied progesterone preparations. (Modified from [30])

Table 4. Progesterone pharmacokinetics during treatment with vaginal suppositories and vaginal gel. (Modified from Duijkers et al. [30])

Parameter	VS 100 mg	VS 200 mg	VS 400 mg	VS 400 mg	VG 90 mg
	2 times a day	2 times a day	2 times a day	1 × daily	1 × daily

C_{max} (ng/mL) First dose	8.37	11.15	11.36	11.63	7.01
$AUC_{0–12\,h}$ (h·ng/mL) First dose	65.67	88.56	92.50	91.10	56.00
C_{max} (ng/mL) Repeated dose [a]	9.59	14.90	19.8	14.8	9.61
$AUC_{0–24\,h}$ (h·ng/mL) Repeated dose [a]	136.39	241.84	365.32	252.86	156.59
VG Vaginal gel, *VS* Vaginal suppository [a] Treatment day 24					

The highest serum progesterone levels are reached after intramuscular administration, while vaginal treatment leads to an increased concentration in the endometrium. Nevertheless, a meta-analysis of 15 randomized controlled trials (RCTs) did not reveal any significant differences between vaginal and intramuscular progesterone treatment for luteal phase support in the context of assisted reproduction with regard to the pregnancy rate and course as well as the rate of miscarriages. However, satisfaction with vaginal treatment with progesterone was significantly higher than with intramuscular application [31].

Another meta-analysis of 8 RCTs with more than 3800 patients, 1523 with dydrogesterone (20–40 mg/day), 1388 with progesterone-containing vaginal capsules (600–800 mg/day), and 898 with progesterone-containing VG (90 mg/day). Treatment of luteal phase support as part of IVF treatment revealed no relevant difference between dydrogesterone and vaginal progesterone in terms of pregnancy maintenance. However, there were indications that dydrogesterone was better tolerated than vaginally administered progesterone [32].

Uterine first pass effect

After the treatment of 14 postmenopausal women the day before a transabdominal hysterectomy at 8:00 a.m. and 6:00 p.m. and the following morning at 6:00 a.m. with 90 mg progesterone as VG or with 50 mg progesterone in the vaginal endometrium in women, the progesterone concentrations were 3 times higher than in the intramuscularly treated patients (1.1 vs. 0.4 ng/mg protein), while the serum levels after intramuscular administration were significantly higher than after vaginal treatment (29.4 vs. 4.8 ng/mL; [33]).

When a uterine perfusion model was examined, ^{3}H-progesterone, which was applied to the vaginal tissue remaining on the uterus after the hysterectomy, diffused through the entire uterus within 4 to 5 hours. Already 4–5 hours after application, progesterone concentrations of 185 ± 155 ng/100 mg in the endometrium and 254 ± 305 ng/100 mg in the myometrium were reached [34].

Transdermal application of progesterone

The daily transdermal application of a cream with 40 mg progesterone led to a very low serum progesterone level of 0.22 ng/mL in women treated transdermally with 50 µg estradiol, which rose to 1.67 ng/mL by day 42 [20].

Even the cyclic 14-day application of a cream with 16, 32, or 64 mg progesterone over 3 cycles did not cause sufficient secretory transformation of the endometrium with a maximum progesterone level of 1.0 ng/mL [20]. Reasons to consider are insufficient estradiol priming, too short a duration of progesterone treatment and/or, most likely, inadequate cutaneous absorption of progesterone. It is possible that the high 5α-reductase activity in the skin inactivates a large part of the absorbed progesterone, so that its antiproliferative effect on the endometrium is not sufficiently effective [20].

However, there are also contradicting data: For example, transdermal application of a cream containing 15 or 40 mg progesterone twice a day in women substituted with 0.625 mg conjugated estrogens resulted in a significant suppression of endometrial proliferation, although the progesterone levels were very low [35]. The extent to which the progesterone metabolite 20α-DHP, which has 25–50% of the progestogenic activity of progesterone, contributes to the suppression of endometrial proliferation has not been clarified. In a placebo-controlled study, the daily application of a cream containing 20 mg of progesterone to the skin of postmenopausal women with vasomotor symptoms led to an improvement or elimination of symptoms in 83% of the patients, while no effects on bone density were found [36].

However, various prospective, randomized double-blind studies have shown that the effects of transdermal treatment with a progesterone-containing cream on hot flashes, libido, bone density, mastodynia, some biochemical parameters, and endometrial proliferation do not differ from those of placebo [37].

Intranasal application of progesterone

By suspending progesterone in almond oil, the bioavailability of progesterone can be increased to 18%. The intranasal application of 11 mg progesterone (= 4 sprays) led to an increase in serum progesterone to 3.8 ng/mL, which, after a temporary decrease, was followed by a second increase to 2.7 ng/mL [38]. The bioavailability of intranasal progesterone can be increased if it is combined with hydrophilic methylated cyclodextrin to form a water-soluble complex.

Subcutaneous or intramuscular application of water-soluble progesterone

Due to the lack of hydrophilic hydroxyl groups, progesterone is hardly soluble in water. This disadvantage can be avoided by combining progesterone with the polysaccharide hydroxypropyl-β-cyclodextrin to form a highly water-soluble complex. After intramuscular or subcutaneous injection of the aqueous solution, the complex disintegrates and the progesterone released can be quickly absorbed. As a result, the bioavailability of progesterone is higher than after the injection of an oily suspension of progesterone.

The preparation with an aqueous solution of 25 mg progesterone was injected sc or im once a day. After subcutaneous administration, the progesterone level rose rapidly to 51 ± 16 ng/mL and then gradually fell to 6.6 ± 1.6 ng/mL [39].

The effects of subcutaneous injection of an aqueous solution containing 25 mg, 50 mg, and 100 mg of progesterone were examined in a pharmacokinetic study. Approximately 1 hour after the application, peak values of 58 ng/mL, 103 ng/mL, and 235 ng/mL were measured—proof of the linear relationship between dose and serum concentration. In general, the water-soluble progesterone supplement was well tolerated; side effects were local reactions at the injection site, especially local edema due to temporary fluid retention [40].

Conclusion for practice

- **In the context of assisted reproduction, progesterone or dydrogesterone is used to support the luteal phase (Table 5).**
- **After *oral* progesterone administration, more than 30 metabolites are formed, which in immunoassays can cause false high results of the systemic progesterone concentration. This problem can only be solved by prior chromatographic separation of the progesterone or by direct measurement methods.**
- **In principle, the following applies: *Parenteral* administration achieves higher serum progesterone levels than oral administration. For this reason, progesterone preparations are only used parenterally in reproductive medicine, which some patients find uncomfortable.**
- **Vaginal treatment with progesterone in the form of suppositories, capsules, or gel is associated with large inter-individual fluctuations.**
- **The highest progesterone concentrations are reached in the serum after intramuscular administration and in the endometrium after vaginal treatment.**
- **In several studies, no effects of transdermal treatment with a progesterone-containing cream were found compared with placebo with regard to various clinical and biochemical parameters.**
- **Determining progesterone in saliva to control progesterone therapy is not advisable due to inaccurate measurement methods.**
- **Dydrogesterone is metabolized only to a small extent after oral administration. Neither dydrogesterone nor its metabolites have any central effects.**

Table 5. Progesterone preparations that are used in Germany for luteal phase support in the context of assisted reproduction. Arranged in alphabetical order

Preparation	Dosage form	Application	Single dose	Daily dose recommended by the manufacturer (only applies to approved preparations)	Approval for the indication luteal phase support within the framework of ART	Storage
Crinone®, 8% vaginal gel (Merck, Darmstadt)	Vaginal gel	Vaginal	90 mg	Once a day from the day of the embryo transfer up to a total therapy duration of a maximum of 30 days	Yes	Not above 25 °C, do not freeze
Cyclogest®, 400 mg vaginal	Vaginal suppositories	Vaginal	400 mg	Twice a day from the day of egg retrieval for	Yes	Not above 30 °C

suppositories (Gedeon-Richter, Cologne)				a maximum of 38 days (corresponds to a maximum of 7 + 2 weeks gestation)		
Duphaston®, 10 mg (Mylan Healthcare GmbH, Troisdorf)	Film-coated tablet	Orally	10 mg [c]	"Off label" 3 times a day from the day of egg retrieval or one day later until at least the pregnancy test	No	No special requirements
Famenita®, 100 mg soft capsules (Exeltis Germany, Ismaning)	Soft capsule	Vaginal [a]	100 mg	"Off label", 2 soft capsules are usually used vaginally 3 times a day, starting with the day of egg retrieval or one day later	No	Not above 30 °C
Famenita®, 200 mg soft capsules (Exeltis Germany, Ismaning)	Soft capsule	Vaginal [a]	200 mg	"Off label", a soft capsule is usually used vaginally 3 times a day, starting on the day of egg retrieval or one day later	No	Not above 30 °C
Lutinus®, 100 mg vaginal tablets (Ferring GmbH, Kiel)	Vaginal tablet	Vaginal	100 mg	3 times a day from the day of egg retrieval until the pregnancy test. In case of pregnancy, continue treatment for another 30 days	Yes	No special storage conditions
Progestan® (Dr. Kade / Besins Pharma	Soft capsule	Vaginal [a]	100 mg	"Off label", 2 soft capsules are usually used vaginally 3	No	Not above 30 °C

GmbH, Berlin)				times a day, starting with the day of egg retrieval or one day later		
Prolutex® (Marckyrl, Papenburg)	Solution for injection	Subcutaneously or intramuscularly	25 mg	Once a day from the day of egg retrieval, up to a maximum of 12 weeks of gestation	Yes	Not above 25 °C, not in the refrigerator, do not freeze
Proluton® Depot, 250 mg ampoules (Bayer Austria Vienna)	Oily solution for injection	Intramuscular	250 mg [b]	"Off label", 1–2 ampoules are usually applied at weekly intervals	No	Not above 25 °C
Utrogest®, 100 mg soft capsules (Dr. Kade / Besins Pharma GmbH, Berlin)	Soft capsule	Vaginal [a]	100 mg	"Off label", 2 soft capsules are usually used vaginally 3 times a day, starting with the day of egg retrieval or one day later	No	Not above 30 °C, not in the refrigerator
Utrogest®, 200 mg soft capsules (Dr. Kade / Besins Pharma GmbH, Berlin)	Soft capsule	Vaginal [a]	200 mg	"Off label", a soft capsule is usually used vaginally 3 times a day, starting on the day of egg retrieval or one day later	No	Not above 30 °C, not in the refrigerator
Utrogest® Luteal, 200 mg soft capsules (Dr. Kade / Besins Pharma GmbH, Berlin)	Soft capsule	Vaginal	200 mg	One soft capsule 2–3 times a day, starting on the day of hCG administration (trigger) and at least up to the 7th week of pregnancy, but	Yes	Not above 30 °C

				no longer than the 12th week of pregnancy		

Not all of the listed products are approved for use under ART. The preparation Duphaston® contains dydrogesterone. The preparation Proluton® Depot contains hydroxyprogesterone caproate, a derivative of 17α-hydroxyprogesterone

ART "assisted reproductive technology", *hCG* human chorionic gonadotropin, week of pregnancy

[a] The approved type of application is oral application, but in the context of ART, these preparations are used vaginally "off label"

[b] Hydroxyprogesterone caproate. Indication according to the technical information: habitual abortion caused by luteum insufficiency

[c] Dydrogesterone. Indications according to the technical information: 1. Treatment of cycle irregularities, 2. As a progestogen as part of a combined hormone replacement therapy

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Compliance with ethical guidelines

Conflict of interest. H. Kuhl and I. Wiegratz state that they have no conflict of interest.

The authors did not conduct any human or animal studies for this article. The ethical guidelines given there apply to the studies listed.

Literature

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