



Nestorone®: clinical applications for contraception and HRT

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Abstract

The 19-nor derivatives of progesterone are referred to as "pure" progestational molecules as they bind almost exclusively to the progesterone receptor (PR) without interfering with receptors of other steroids. In this category is Nestorone®, which has strong progestational activity and antiovaratory potency with no androgenic or estrogenic activity *in vivo*. These properties make it highly suitable for use in contraception and hormonal therapy (HT). Due to its high potency, very low doses of Nestorone may be delivered via long-term sustained-release delivery systems. Nestorone, 75 or 100 µg per day, released by vaginal ring has suppressed ovulation in women, with inhibition of follicular maturation. A vaginal ring releasing both 150 µg of Nestorone and 15 µg of ethinyl estradiol per day has effectively suppressed ovulation for 13 consecutive cycles. Nestorone has also been used effectively in a single implant for contraception in breastfeeding women and shows promise for use in transdermal systems as a contraceptive or for HT when combined with estrogen.

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1. Introduction

The variety of progestins synthesized in the last decade differ in progestational potency, pharmacokinetic properties and other effects related to their source molecules. Important among these are the 19-nor derivatives of progesterone, which are pure progestational molecules that bind almost exclusively to the progesterone receptor without interfering with receptors of other steroids [1]. One of the most potent progestins is Nestorone® (16-methylene-17 α -acetoxy-19-norpregn-4-ene-3,20-dione),¹ a 19-nor derivative that has progestational potency 100 times greater than progesterone and 10 times greater than levonorgestrel (LNG) [2]. Nestorone's 19-methyl substitution and the addition of the 16-methylene substituent, which enhances binding to the progesterone receptor, contribute to the molecule's high progestational activity [3,4]. Nestorone is not active orally; however, when given parenterally it requires small amounts to achieve efficacy, and is thus well suited for use in long-term sustained-release delivery systems, such

as implants, vaginal rings, and transdermal methods (e.g. patch or gel).

2. Classification of progestins

Many progestins currently on the market or in development are derived from testosterone (19-nortestosterone) or from progesterone, either 17-OH progesterone or 19-norprogesterone (Table 1). Most available oral contraceptives are 19-nortestosterone derivatives and have some androgenic activity. The first generation progestin of the 19-nortestosterone derivatives is norethynodrel, the first progestin synthesized. The second generation progestins are classified into the estrane group (norethisterone or NET and metabolites) or the gonane group (LNG and derivatives). The third generation progestins (LNG derivatives) are desogestrel, etonogestrel (3-ketodesogestrel), gestodene, and norelgestromin and are less androgenic than second generation progestins [5]. Dienogest is a recently synthesized molecule that is a hybrid derived from the estrane group, and has a 17 α -cyanomethyl group. Unlike most other 19-nortestosterone derivatives, dienogest is antiandrogenic [6].

The 19-norprogesterone derivatives, while binding more specifically to the progesterone receptor, do not bind to

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¹ Nestorone® is the registered trademark of the Population Council for the steroid 16-methylene-17 α -acetoxy-19-norpregn-4-ene-3,20-dione.

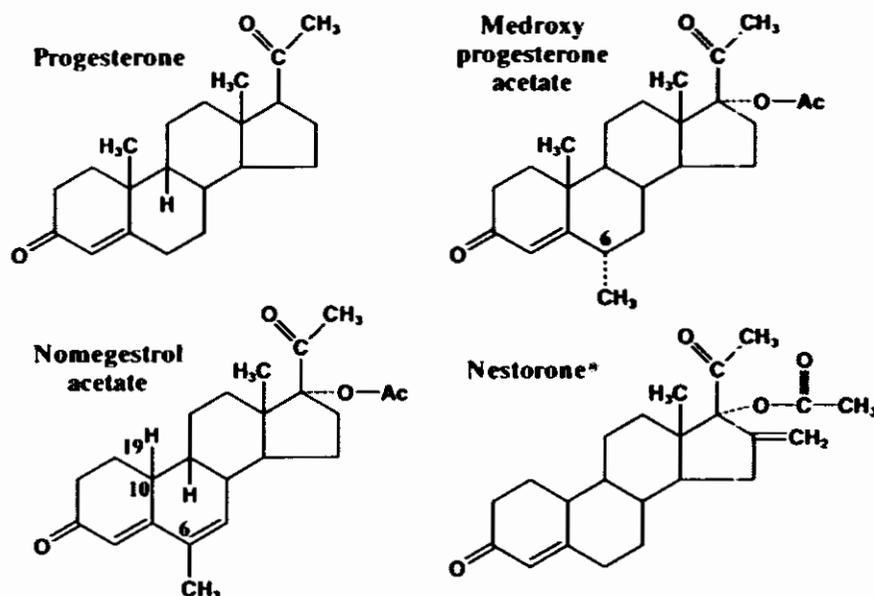


Fig. 1. Chemical structures of progesterone and three progesterone derivatives.

androgen or estrogen receptors. Fig. 1 demonstrates the chemical structure of progesterone and several progesterone derivatives. Besides Nestorone, other 19-norprogesterone derivatives are demegestone, promegestone (R5020), trimegestone (TMG), and nomegestrol acetate (NOM Ac) [1].

The 17-OH progesterone derivatives have varying activities [5] and include: medroxyprogesterone acetate (MPA), which exerts some androgenic and glucocorticoid actions at high doses; megestrol acetate; cyproterone acetate (CPA), the most potent antiandrogen; and chlormadinone acetate. Drospirenone, a new progestin, is derived from spiro lactone and has activity similar to progesterone itself [7].

Receptor binding studies and *in vivo* bioassays have been performed to compare the progestational, androgenic and estrogenic properties of Nestorone with other selected progestins.

Table 1
Classification of synthetic progestins

Related to progesterone	Related to testosterone
17-OH-progesterone	Estranes
Medroxyprogesterone Ac	Norethisterone
Cyproterone Ac	Dieneogest
Chlormadinone Ac	Gonanes
Megestrol Ac	Levonorgestrel
Medrogestone	Desogestrel
19-Norprogesterone	Etonogestrel
Demegestone	Gestodene
Promegestone	Norelgestromin
Nestorone	
Nomegestrol Ac	
Trimegestone	
Spirolactone derivative	
Drospirenone	

3. Activities of Nestorone®

3.1. Progestational activity

3.1.1. PR binding studies

Steroid receptor binding studies [2] have compared the relative binding affinity of progesterone, Nestorone, 2-ketodesogestrel (etonogestrel), and LNG to progesterone receptors (PR) using rat uterine cytosol preparation. The relative binding affinities from highest to lowest were as follows: 3-ketodesogestrel (220), Nestorone (110), LNG (100) (reference compound), and progesterone (20) [2]. Another study has shown the relative PR binding affinity of a new progestin, the 18-methyl analog of Nestorone, to be three times greater than that of Nestorone or LNG [8].

3.1.2. *In vivo* bioassays for progestational activity

Progestational activity of Nestorone versus LNG and progesterone was assessed using three different assays [2,8]: (1) On the endometria transformation test in immature female rabbits primed with estradiol (E_2) dose-dependent increases in uterine weight and the McPhail Index were observed for the three progestins administered subcutaneously (s.c.). Nestorone exhibited 10 times greater potency than LNG and was 100 times more potent than progesterone on this test. Nestorone was also 100 times more potent when administered s.c. than when given orally (2). The pregnancy maintenance test in ovariectomized rats showed that the following minimal s.c. doses per rat per day were effective in maintaining pregnancy: 0.03 mg of the new Nestorone analog; 0.3 mg of Nestorone; 0.3 mg of LNG; and 5 mg of progesterone. (3) Using the ovulation inhibition assay in cycling rats, dose-dependent inhibition of spontaneous ovulation was observed for each progestin tested (Nestorone.

LNG and P) when administered s.c. Nestorone was the most potent of these progestins, completely inhibiting ovulation at a dose of 10 µg per rat per day versus 20 µg LNG and 900 µg progesterone [2].

Nestorone and its 18-methyl analog are among the most potent progestins when compared to other new molecules. Tests of the progestational potency of additional progestins have shown that NET, MPA and drospirenone are more potent than P when given orally [5,7] but are less potent than LNG; NOM Ac is four times more active than MPA [9], whereas trimegestone, a recently synthesized progestin, is 200 times more potent than progesterone, and hence twice as potent as Nestorone [10].

3.2. Androgenic activity

3.2.1. AR binding studies

Relative binding affinity to androgen receptors, using the rat ventral prostate as a source, was as follows: testosterone (100), LNG (70), 3-ketodesogestrel (40), Nestorone (0.2). 18-Methyl-Nestorone, likewise, showed almost no binding to AR [2,8].

3.2.2. In vivo androgenic activity

The weights of the ventral prostate and levator ani of immature castrated male rats were compared after treatment with either the control (testosterone) or with 3-ketodesogestrel, LNG, or NES. Nestorone at doses of 1 and 4 mg per day had no effect on these models; LNG was more potent than 3-ketodesogestrel, and T was two- to three-fold more potent than these two progestins, respectively. Both LNG and 3-ketodesogestrel exhibited a dose-dependent weight increase of the ventral prostate. Nestorone had no effect on LH in castrated male rats, while LNG and T suppressed LH in a dose-dependent manner. The data showed that Nestorone has no androgenic or antiandrogenic activity when administered at a dose of 20 mg/kg per day, which far exceeds the effective dose for contraception in humans [2].

3.3. Estrogenic activity

3.3.1. ER binding

Neither Nestorone nor any other of the progestins studied by Kumar et al. exhibited significant binding to estrogen receptors using immature female rat uteri [2].

3.3.2. In vivo estrogenic activity

The uteri of immature ovariectomized female rats treated s.c. with Nestorone or LNG were weighed to assess the in vivo estrogenic effect of these progestins. Levonorgestrel at 1 and 5 mg per day was associated with significant increase in uterine weight, while Nestorone at similar doses had no uterotrophic activity. LNG plus estradiol had a synergistic effect on uterine weight increase; however, Nestorone did not affect the uterotrophic activity of estradiol.

Both LNG and Nestorone exhibited antiestrogenic activity in that both blocked vaginal cornification induced by E₂ [2].

3.4. Glucocorticoid activity

Several progestins bind to the glucocorticoid receptors. Nestorone, as well, has exhibited binding affinity to calf thymus glucocorticoid receptors; however, neither Nestorone nor LNG had glucocorticoid-specific responses in rat liver, e.g. glycogen and tyrosine transaminase production [2]. On the other hand, Nestorone exhibits thymolytic activity in rats at very high doses (a dose 2000 times greater than the dose effective in inhibiting ovulation in rats) (Population Council, data on file). Medroxy progesterone acetate is another progestin exhibiting glucocorticoid activity at the high doses used for cancer indications [11,12], while megestrol acetate shows 50% less activity than MPA [13]. None of these progestins exert actions at the therapeutic doses used for contraception or hormone therapy.

4. Pharmacokinetics of Nestorone

Nestorone is not active orally, but continuous delivery in sustained-release implants, vaginal rings or intrauterine systems makes the molecule more available to target tissues.

Nestorone, like progesterone, does not bind to SHBG and has a shorter half-life and higher clearance rate than progestins that exhibit SHBG binding [14]. Nestorone's lack of SHBG binding and its large volume of distribution is consistent with its high affinity for PRs and its accumulation in extravascular space [15]. The free fraction of Nestorone and other progestins (e.g. progesterone and dienogest) that do not bind to SHBG [6] should be greater than most T-derived progestins that bind to SHBG.

Noé et al. [15] have studied the pharmacokinetics of Nestorone when administered orally, by i.v. bolus injection or via implant. The two-component half-life of Nestorone after an i.v. bolus injection (100 µg dose) is 3.5 ± 0.5 min and 83 ± 14 min. The volume of distribution of Nestorone is 4.7 ± 1.3 l/kg, its metabolic clearance rate is 6 l/kg per day and bioavailability after oral administration (100 µg dose) is 10% of the dose [15]. When tritiated Nestorone was administered orally, 43% of radioactivity was recovered from the urine.

Noé et al. [15] also found that the clearance rate of Nestorone is slower after long-term use of subdermal implants. In this study of five women each implant had an initial load of 78 mg of Nestorone and released on average 100 µg per day. Upon implant removal, Nestorone levels decreased to 50% at 180 min (~45 pmol/l) after the highest level was detected, with no change in levels until 480 min.

Lähteenmäki et al. [16] found great interindividual variation in Nestorone levels within implant treatment groups.

although a linear correlation between mean NES plasma levels and implant length was observed; 39 subjects were implanted with NES capsules of either 30, 15 or 7.5 mm in length (drug loads of 40, 20 or 10 mg, respectively). Two- to three-fold differences in NES plasma concentrations were observed among subjects receiving the same daily doses, which the authors suggest may be due to individual differences in hepatic blood flow rates and metabolic capacities.

5. Nestorone in long-acting systems for contraception and hormone therapy

Nestorone is presently under investigation for use in a variety of sustained-release delivery systems, such as implants, vaginal rings, and transdermal patch or gel formulations, for contraception or hormone therapy. These novel delivery systems avoid the first pass effect on the liver that occurs with oral intake and provide steady serum steroid levels without the fluctuations observed with oral contraceptives. They are either user-controlled or require no attention and thus generally have high compliance rates. Potent progestins, such as Nestorone, that are not orally active are well suited for use in sustained-release delivery systems, which necessitate use of low steroid doses. The unique features of Nestorone are listed in Table 2.

5.1. Nestorone delivered from vaginal rings

Recent technological advances have enhanced the delivery capabilities of vaginal rings that release a single progestin or an estrogen-progestin combination for use in contraception and hormone therapy. A number of studies with different steroid-releasing rings have been conducted [17-27], and two rings have been FDA-approved to date: Estring[®], releasing estradiol for hormone therapy and NuvaRing[®] releasing etonogestrel and ethinyl estradiol (EE) for contraception. Studies of different formulations of Nestorone-containing vaginal rings for contraception are ongoing and include a Nestorone-only ring and a combination ring releasing Nestorone and EE. The vaginal ring used in current studies is made of dimethylsiloxane/vinylmethylsiloxane copolymer and is approximately 56 mm in overall diameter and 8.4 mm in cross-section. The steroid(s) are contained in cores within the ring body and diffuse at a slow, steady rate. The rings are designed for 1-year efficacy.

Table 2

Nestorone: a progestin well suited for sustained-release delivery

High progestational potency
Not active orally
Not bound to SHBG
No androgenic or estrogenic effects
High antiovaratory effect

5.1.1. Nestorone vaginal ring

A Nestorone-only vaginal ring has been evaluated for contraception in normal cycling women and in lactating women. A 6-month study of a vaginal ring delivering either 50, 75 or 100 µg per day of Nestorone has been completed. Of 180 women enrolled, two pregnancies were reported in the 75 and 100 µg per day ring groups, both during the fourth month of use. Mean bleeding-free days decreased with time, ranging from 64 to 70 and 53 to 77 during the first and second trimesters, respectively (~90 days per trimester). Days of bleeding and spotting were lowest with the 100 µg per day ring. The cumulative continuation rates by dose at 6 months were 84.8, 76.7 and 73.2%, respectively (Population Council, data on file).

Serum levels of Nestorone remained constant at 125, 200 and 250 pmol/l for the 50, 75 and 100 µg per day dose groups, respectively [26]. Percentage of sampling periods with ovulation inhibition ($P < 10$ nmol/l) was 97.5, 97.4 and 98.8% in the 3 dose groups, respectively (Fig. 2). Although no difference was observed in luteal activity between the two lower-dose groups, results suggest higher efficacy with the higher dose.

Ovarian function was also assessed by measurements of serum E₂ levels. The highest E₂ levels inversely correlated with NES dose. Estradiol levels were high and variable according to the month of sampling with use of the low-dose ring. E₂ levels <100 pmol/l occurred only in the high-dose group in 5% of the sampling runs; E₂ levels below 350 pmol/l occurred in 25, 56 and 70% of subjects in the low-, mid- and high-dose groups, respectively. More pronounced inhibition of follicular maturation was observed with the 75 and 100 µg per day rings; E₂ levels in the higher dose groups remained in the range of 300-400 pmol/l, which is characteristic of early follicular phase secretion. The three rings effectively inhibited ovulation and suppressed luteal activity with Nestorone serum levels >100 pmol/l.

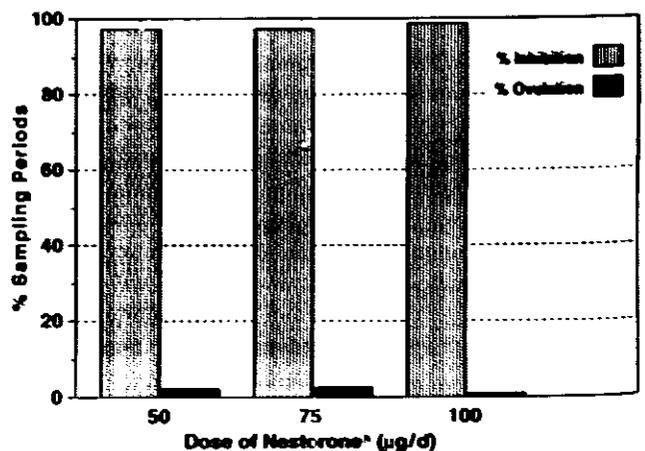


Fig. 2. Percent of ovulation inhibition ($P < 10$ nmol/l) in sampling periods with use of the Nestorone-only vaginal ring releasing either 50, 75, or 100 µg per day.

These results indicate that Nestorone is highly effective in preventing follicular growth and ovulation at doses of 75 and 100 μg per day. The later dose induced the higher rate of ovulation inhibition (only 1.2% of samples indicated luteal activity) and the lower number of bleeding and spotting days over 6 months of use.

Massai et al. [21] studied the 50 μg per day Nestorone-only ring for contraception during the postpartum period in 50 lactating women. No pregnancies occurred in 555 women-months of observation during the 1-year study period. Nestorone serum levels, initially around 127 pmol/l, remained constant at approximately 74 pmol/l until the end of the study. Because Nestorone is orally inactive it did not affect the nursing infants; infant growth and breastfeeding performance did not differ from that of infants whose mothers were using the copper IUD in another study. During the study about 80% of women became amenorrheic, a rate that fell to about 50% at 1 year. Lactational amenorrhea lasted for a mean of 319 days.

5.1.2. NES/EE ring: dose-finding study

In a dose-finding study conducted in 5 clinics, 152 normal cycling women, used the NES/EE contraceptive ring for 1 year (Fig. 3). Each ring contained one of three Nestorone/EE dosage ratios, 150/15, 150/20 or 200/15 μg per day, and was used on a schedule of 3 weeks in/1 week out. The objective of the study was to determine the effectiveness of the three dose ratios in preventing luteal activity and in controlling menstrual bleeding patterns [28].

The three dosages inhibited ovulation in the majority of subjects. However, two pregnancies did occur, both in the 200/15 μg per day group. In the 150/15 group, luteal activity, defined as serum progesterone values ≥ 10 nmol/l, was reported in 8 (4 cycles in 2 women weighing >100 kg) of 120 cycles, in the 200/15 group in 4 of 118 cycles and in the 150/20 group in 0 cycles.

The ring provided good bleeding control with regular bleeding during the week of ring withdrawal; in all subjects breakthrough bleeding or spotting occurred on approximately 1.4 to 1.5% of days while the ring was in place.

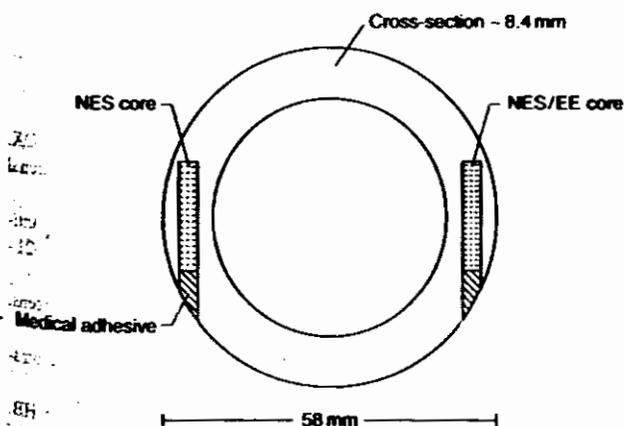


Fig. 3. Cross-section of the Nestorone/ethinyl estradiol contraceptive ring.

Most discontinuations were due to use or device problems, personal reasons or other nonserious adverse reactions. One serious adverse event, possibly related to ring use and resulting in early termination, was a superficial thrombophlebitis.

Lipid measurements compared before and 1 year after ring use showed that HDL values increased 23–38%, while LDL declined 3–7%. Increases in total cholesterol (3–11%) and in triglycerides (7–11%) were also noted.

Colposcopic examination of subjects' vaginas and cervixes indicated no device-related changes. These findings corresponded to results of Fraser et al. [29], who found no significant changes in the vaginal epithelium upon colposcopic examination in users of various ring formulations, including a Nestorone-only ring and a Nestorone/EE ring.

In summary, Nestorone used in combination with EE in vaginal rings has performed well with respect to ovulation inhibition and control of bleeding patterns without serious device-related effects.

5.2. Nestorone delivered in a single subdermal implant

Contraceptive systems using multiple implants, such as Norplant[®] and Jadelle[®], have been available to women for several years. Refinements in implant technology and the availability of potent progestins have allowed for the development of single implant systems. With use of potent progestins, a single implant system may achieve contraceptive efficacy and is likely to be more acceptable to users.

A single implant releasing Nestorone alone has been tested in lactating women in a 2-year study comparing the Nestorone implant to the copper T intrauterine device (T-Cu) [30]. Nestorone is inactive orally and thus will not affect the nursing infant. The implant, which is 4 cm in length and has a silicone drug matrix core containing 80 mg of Nestorone, initially releases, *in vitro*, about 100 μg per day. The mean Nestorone serum levels were 175 pmol/l at 1 month postinsertion; by the end of the first year, Nestorone levels had decreased to 60 pmol/l. Nestorone concentrations in the breast milk were 54–135 pmol/l.

No pregnancies occurred in 2195 and 2145 women-months of exposure to the Nestorone implant and the T-Cu, respectively. During lactation and in the first 6 months of the study Nestorone implant users had significantly less irregular bleeding than did the T-Cu users ($P < 0.002$). Implant users exhibited significant increase in irregular bleeding after infant weaning ($P < 0.05$), similar to that experienced by T-Cu users. No serious adverse events that were likely to be related to Nestorone implant use were observed in either group.

These results suggest that Nestorone delivered in a single implant provides effective alternative contraception for 2 years during lactation and after weaning and represents a promising method of long-acting birth control [30].

5.3. Nestorone delivered from transdermal systems

Progestins used in a transdermal patch or gel must be effectively absorbed through the skin and must be able to achieve a systemic effect in small doses. Progesterone was first used in a transdermal hydroalcoholic gel. After application to the skin, very low plasma levels insufficient to achieve systemic efficacy were detected, because the steroid was converted in the skin via the enzyme 5α reductase to an inactive metabolite (5α dihydro progesterone) [31]. Other progestins, such as Nestorone, trimegestone, norethisterone acetate, levonorgestrel, norgestimate and its active metabolite norelgestromin, do not undergo this conversion and are able to achieve higher systemic levels when applied transdermally.

Although inactive orally, Nestorone is particularly well suited for transdermal delivery because it is highly active when applied to the skin and achieves good systemic bioavailability. Due to its high progestational potency and anti-ovulatory effects, Nestorone, when absorbed transdermally can achieve systemic serum levels that suppress ovulation in the majority of subjects ([32], Population Council, data on file). In a 3-month multicenter study of 150 normal cycling women, Nestorone gel was applied to abdominal skin in daily doses of 0.3, 0.6 and 1.2 mg. Results showed a clear dose–response effect in serum Nestorone levels achieved and in ovulation inhibition: 53, 64 and 83% inhibition in the 3 dose groups, respectively.

Of the progestins tested for transdermal hormone therapy, Nestorone and trimegestone are the most suitable compounds; they rate among the most potent progestational molecules and have proven to be active at very low doses. The publication of the Women's Health Initiative (WHI) study results in July 2002 [33] has increased interest in the effects of other molecules and other routes of administration of sex steroids for hormone therapy. Given its pharmacologic profile, the safety and efficacy of Nestorone delivered transdermally with or without estrogen deserves further research.

6. Conclusion

Nestorone, a 19-norprogesterone derivative, is one of the most potent progestins developed to date. It exerts a high antioovulatory effect at low doses and appears to be active in women when released from subdermal implants at a dose of 100 μ g per day and from vaginal rings at 75 and 100 μ g per day. Although results of the WHI study [33] have raised many questions regarding the safety of progestins, a large body of evidence indicates that nonandrogenic progestins induce fewer metabolic changes and side-effects. The pharmacological profile of 19-norprogesterones in general, and of Nestorone in particular, renders this class of compounds suitable for contraception and hormone therapy and warrants further research in these areas.

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