Progestin use in mares

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Abstract

Progesterone is in constant fluctuation throughout the lifespan of the horse. As a key regulator of the hypothalamic-pituitary-gonadal axis, progesterone is involved in numerous aspects of reproduction. Synthetic analogs of progesterone, deemed progestins, are widely available to industry to act as progestogenic within the reproductive tract, although few are bioactive in the horse. Utilized to suppress estrous, delay ovulation, maintain pregnancy, and for behavioral modifications, progestins are one of the most common class of pharmaceuticals in veterinary medicine. In this review, we discuss the progestins available to equine industry, their efficacy, pharmacokinetics, and potential side effects following treatment.

Keywords: Progestin, estrus synchronization, delay of ovulation, pregnancy maintenance

Introduction

Many progestins have been investigated in equine veterinary medicine, including medroxyprogesterone acetate, hydroxyprogesterone hexanoate, norgestomet, megesteral acetate, melengestrol acetate, and proligestone (Table). Several studies[1-5] investigated the progestogenic efficacy of these progestins and determined only altrenogest to be effective. Altrenogest maintained pregnancy after a luteolytic dose of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), whereas medroxyprogesterone acetate (MPA), hydroxyprogesterone hexanoate, norgestomet, and megesteral acetate treatment were unable to prevent abortion.[1] Furthermore, the ability of proligestone, MPA, and melengestrol acetate to bring mares out of seasonal transition was not consistent.[2-4] In contrast, both progesterone in oil[2] and altrenogest[5] effectively hastened estrus and conception in both early and late seasonally transitional mares. Therefore, this review will focus on altrenogest, with minimal inferences to other progestins.

Pharmacokinetics of altrenogest

Altrenogest (17_{α}-allyl-17_{β}-hydroxy-estra-4, 9, 11-trien-3-one) is also known as allyltrenbolone. Although classified as a progestin, altrenogest structurally resembles androgens and binds to the androgen receptor with 75% affinity of dihydrotestosterone,[6] although minimal anabolic effects have been noted, including no increase in body mass in either intact stallions or mares.[7] However, mares treated with altrenogest in late pregnancy had fillies born with an enlarged clitoris (Figure 1).[8] Trace concentrations of androgen and anabolic steroid trenbolone were noted in horses receiving altrenogest, leading to restrictions of its use in many equine organizations, including Irish Horseracing Regulatory Board, British Horseracing Association, Australian Racing Board, and Federation Equestre Internationale.

To predict withdrawal times for altrenogest, its pharmacokinetics were determined after oral, injectable, and rectal treatments.

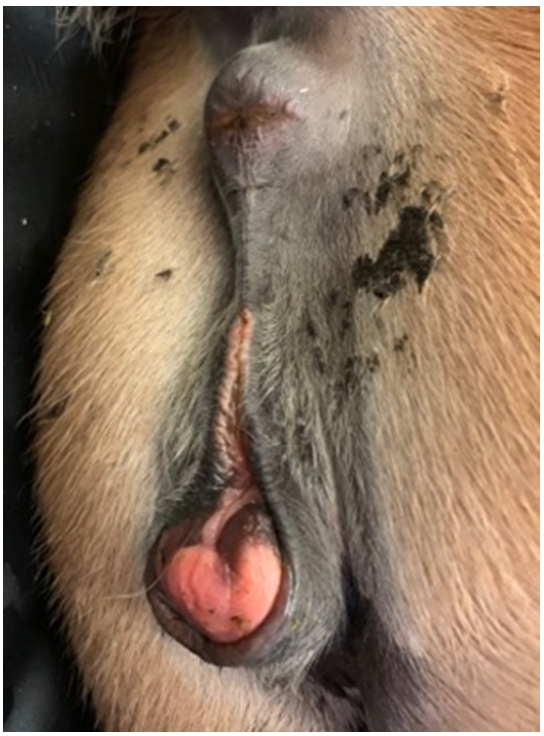

Figure 1. Anabolic effects (enlarged clitoris) of altrenogest treatment during late pregnancy

Adult mares (n = 10) were treated with oral altrenogest (0.044 mg/kg).[9] In most mares, maximum concentration (C_{max}) was reached within 15 - 30 minutes. Mean C_{max} was 35 ng/ml on day 1 and 31 ng/ml on day 5. In contrast, mean C_{max} in urine was 1720 ng/ml on day 1 and 2107 ng/ml on day 5. Time for total clearance (with a limit of detection of 2 ng/ml) was 12 days.

Due to risk of human interaction with oral altrenogest, injectable forms have gained popularity within industry, and the pharmacokinetics of which have been investigated.[10] After intramuscular treatment (0.3 mg/kg), mean circulating concentrations were 33.52 ng/ml. Additionally, the area under the curve for intramuscular treatment was 5.6-fold higher than the oral product. It was hypothesized that intramuscular treatment formed a depot that slowed absorption, prolonging clearance compared to oral treatment.

Several pregnant mares that are admitted to the hospital for medical or surgical events may be prohibited from oral medications. Therefore, pharmacokinetics of intrarectal treatment of altrenogest have also been assessed. Altrenogest was detected in circulation as early as 15 minutes after oral and rectal treatment (0.088 mg/kg).[11] However, the C_{max} for per rectal altrenogest was considerably lower than that of oral treatment (2.54 versus 16.00 respectively), and clearance of altrenogest was more rapid in the rectal treatment group. Although rectal treatment was effective in delivering altrenogest, the bioavailability was only 5.47% compared to oral treatment. Despite the option that rectal treatment may be a viable alternative for hospitalized mares, a dosage of 0.088 mg/kg every 4 - 8 hours would be necessary to maintain therapeutic concentrations.

Use of progestins

Suppressing estrus

Suppression of estrus in sport and race mares is desirable and progestins are commonly used to accomplish this goal. Progestin treatment suppresses estrus in cycling mares through its high affinity for progesterone receptor (PR) resulting in endometrial and myometrial environments similar to a pregnant mare. Progesterone in oil (100 mg) effectively suppressed estrus and mares returned to estrus within 3 - 4 days after cessation of treatment.[2] Repeated treatment with progesterone (either intramuscular or repositol) led to its accumulation within the system that persisted for > 10 days after the last treatment.[12]

Daily oral treatments with 0.044 mg/kg altrenogest was also sufficient to bind to the PR to suppress estrus.[13] Similarly, compounded long-acting injectable altrenogest formulations suppressed behavioral estrus when used as recommended by the manufacturers.[10,14] Furthermore, long-term altrenogest treatment did not affect future fertility potential[15] and high-dose and prolonged altrenogest treatment did not affect overall hematologic or biochemical properties of the mare.[16] In contrast, Norgestomet, MPA, hydroxyprogesterone caproate, and megesterol acetate had no effect on estrus suppression[17-20] To our knowledge, no other synthetic progestins have been critically evaluated for their ability to suppress estrus-like behavior.

Delaying ovulation

Although select progestins successfully suppressed estrus in the mare, conflicting results exist for the ability to delay ovulation, and success may depend on the phase of the estrous cycle in which treatment is initiated. In one study, neither oral altrenogest nor a progesterone implant (controlled intravaginal releasing device) were effective in preventing ovulation when short-term treatment began in the preovulatory phase.[21] In contrast, short-term treatment of 0.044 mg/kg altrenogest successfully delayed ovulation when treated at the detection of a 35 mm follicle.[22] When a double dose of altrenogest (0.088 mg/kg) was administered, the delay of ovulation was noted in a preovulatory mare.[23] However, this was not repeatable in a larger field setting.[1] Interestingly, use of an injectable sustained-release vehicle with varying doses of altrenogest delayed ovulation substantially.[24] The effect was profound when lactide-glycolide microparticles were added, delaying ovulation by 33.5 days after $PGF_{2\alpha}$ treatment. However, MPA had no effect on ovulation when combined with the same vehicle or microparticle formulation.

Hastening transition

Mares are seasonally polyestrous long-day breeders and are therefore anovulatory in the months surrounding the winter equinox. A transitionary phase exists between anestrus and proper cyclicity; and shortening this phase has been attempted through the administration of exogenous steroids. The proposed rational for progestin treatment is believed to inhibit the release of luteinizing hormone (LH) from the pituitary, allowing for a repositol of the gonadotropin to form, thereby allowing for ovulation to be induced. Progestin treatment has been shown to hasten the first ovulation of the year dependent on the stage of transition and the specific progestin used. Altrenogest hastened the occurrence of first estrus and reduced the interval to conception in late transitional mares (> March 15, 20 mm follicle) with no impact on early transitional mares (< March 15th, 14 mm follicle).[5] However, when a higher dose (0.22 mg/kg) was used, results were not repeatable.[25] Other progestins (1500 mg of proligestone) induced comparable ovulation and pregnancy rates to altrenogest treatment in transitional mares.[13] It should be noted that only a minimal number of control mares were used in this study, and pretreatment follicular status was not assessed. Controlled release of intramuscular progesterone (LA P4; BioRelease) was effective in shortening the duration to first ovulation in late transitional mares; however, it was ineffective in the early transitional mare.[26] Studies using intravaginal progesterone-releasing devices to hasten first ovulation had mixed results. Although controlled intravaginal releasing devices combined with progesterone were ineffective in improving pregnancy rates in the first estrous cycle,[27] 89% of anestrous mares ovulated within 10 days after progesterone-releasing

intravaginal devices were remove.[28] It should be noted that mild to moderate vaginitis was observed in a subset of mares after implantation of the progesterone-releasing devices. These devices are not approved for equine use in most countries (except for Cue-Mare® in New Zealand). Overall, the efficacy of progestin treatment in shortening the duration to first ovulation is inconsistent and depends on the reproductive status of the mare in addition to the progestin used.

Synchronizing estrus

For simplicity of breeding in addition to timing embryo transfer, progestins are commonly used to regulate both estrus and diestrus phases of the cycle. Although progestins suppress the release of LH, their effect on FSH is less evident, resulting in normal follicular development.[29] Hence, progestin treatment results in a reduction of ovulations, but does not synchronize follicular growth in treated mares. In order to effectively synchronize estrus in mares, a combination of progestins and estrogens can be used. This 'P&E' treatment consists of 10 days of intramuscular treatment of 150 mg progesterone in oil alongside 10 mg estradiol-17_{β} (Figure 2). This was followed by a luteolytic dose of $PGF_{2\alpha}$ on the 10th day of treatment.[30] It is expected that mares will have a preovulatory follicle 8 days after cessation of treatment and respond to an ovulatory-inducing agent. This has been repeated by replacing progesterone in oil with altrenogest, and responses were comparable.[31]

Supporting early pregnancy

Progesterone is produced in ovaries (primary and secondary corpora lutea) after ovulation until 120 - 150 days of pregnancy when pregnancy becomes fully dependent on 5_{α}-reduced progestogens from the feto-placental unit, including 5_{α}-DHP and 20_{α}-5P.[32-35] Secondary luteal deficiency, as a result of endogenous release of $PGF_{2\alpha}$, is associated with systemic or uterine inflammation and endotoxemia. This premature release is detrimental to pregnancy if it occurs prior to this shift in progestogen release. Although low systemic progesterone concentrations are incompatible with maintenance of pregnancy, there are limited scientific data to support a primary failure of the corpus luteum (CL) as a cause of embryonic loss.[36] Measuring circulating progesterone concentrations in a single blood sample to determine if pregnancy is at risk is not accurate unless it is less than 1 ng/ml, due to variations in secretion of this hormone during the day.

Studies have indicated the progestogenic potential of altrenogest, while no other synthetic progestin has been found as effective. Either 150 mg progesterone in oil or 0.044 mg/kg altrenogest maintained pregnancy after a luteolytic dose of $PGF_{2\alpha}$ in ovariectomized mares.[37] Interestingly, a double dose of altrenogest (0.088 mg/kg) was more effective in pregnancy maintenance in ovariectomized recipient mares after embryo transfer.[38] In this study, it was determined that the standard dose of 0.044 mg/kg produced poor uterine tone and decreased cervical competence.

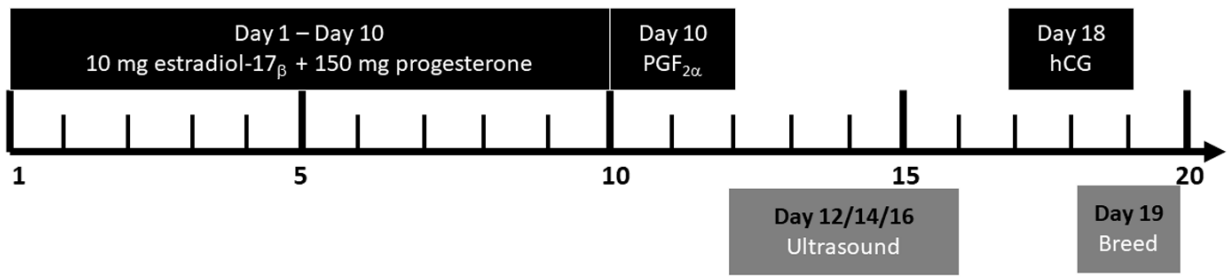

Figure 2: Standard progesterone and estrogen 'P&E' treatment protocol (daily 150 mg progesterone in oil or 0.044 mg/kg altrenogest with 10 mg estradiol-17_{β}). Treatment is initiated when a 25 mm follicle is detected. A luteolytic dose of $PGF_{2\alpha}$ is given on day 10 and transrectal ultrasonography is used to assess follicular development. When a 35 mm follicle (6 - 9 days after cessation of treatment) is detected, hCG is given and mares are inseminated 24 - 36 hours later.

The authors recommended the double dose to be utilized during embryo transfer procedures. Several other synthetic progestins (1000 mg hydroxyprogesterone caproate, 1000 mg MPA, 500 mg hydroxyprogesterone hexanoate, 15 mg norgestomet, and 500 mg megestrol acetate) were unable to maintain pregnancy.[39] Mares treated with altrenogest maintained pregnancy after $PGF_{2\alpha}$ treatment whereas other progestins were not able to prevent abortion.[1] In addition, long-acting progesterone (150 mg) compounds were effective in preventing early pregnancy loss after a luteolytic dose of $PGF_{2\alpha}$ and are worth considering.[40]

There is no cross reactivity between altrenogest and progesterone in commercial progesterone assays; therefore, progesterone assays are helpful in determining whether a CL has undergone luteolysis (P4 < 1 ng/ml) or is still active during treatment. Assay results can guide the clinician to make a decision (discontinue or continue treatment) until the feto-placental unit provides necessary progestogen support to maintain pregnancy. However, altrenogest treatment can suppress endogenous production of progesterone and may interfere with formation of secondary CL.[41,42] One study found altrenogest supplementation to decrease the production of equine chorionic gonadotropin (eCG) in mares greater than 8 years of age, but also found altrenogest treatment to maximize embryo size in the aged mare population.[43]

Supporting late pregnancy

Treatment with a double dose of altrenogest (0.088 mg/kg) prevented late-term abortion in mares after abortion was attempted with a synthetic prostaglandin from 90 - 115 days of pregnancy.[44] Progestins have been suggested to maintain pregnancy in late pregnancy by inducing myometrial quiescence and suppressing $PGF_{2\alpha}$ secretion, presumably by modulation of cytokines. Therefore, altrenogest is routinely used during pregnancy for the prevention or treatment of ascending placentitis, a leading cause of infectious abortion in North America. Combined treatment of antimicrobial (trimethoprim sulfamethoxazole), immunomodulator (pentoxifylline), and progestin (altrenogest) was effective in treating experimentally induced disease (transcervical inoculation with *Streptococcus zooepidemicus*).[45] Although none of the untreated mares carried to term, 10/12 (83%) of the treated mares produced a viable foal. Since treatments were not assessed individually, it is unclear if altrenogest treatment alone prevented abortion. This confers with recent research that demonstrated a withdrawal of PR preceded premature parturition in experimentally induced disease, indicating that altrenogest supplementation may be of no benefit.[46] This was further supported by a study[47] that demonstrated that addition of altrenogest treatment had no effect on pregnancy length, interval from inoculation to delivery, or neonatal outcome in comparison to mares solely treated with antimicrobials and antiinflammatories. In that study, the addition of estradiol cypionate to antimicrobial/antiinflammatory treatment increased pregnancy length from induction of disease, in addition to improving neonatal viability. Furthermore, altrenogest treatment has been shown to shorten pregnancy length.[48] Altrenogest supplementation was also unnecessary in pregnancy maintenance after late ovariectomy (> 100 days), regardless of concentration or time of treatment.[49]

There are some discrepancies with regard to safety of altrenogest treatment in mid- to late-pregnancy. Mares treated with altrenogest in late pregnancy had shorter gestation, poorer neonatal outcomes,[48] and fillies were born with an enlarged clitoris.[8] Additionally, after treatment in late pregnant mares, altrenogest was detectable within both fetal fluid compartments and in grossly higher concentrations in fetal compared to maternal serum.[50] Yet, foals born to mares receiving altrenogest in late pregnancy had normal onset of puberty, comparable fertility, and reproductive function to controls.[51,52] Based on these findings, the efficacy and necessity of progestin supplementation in mid- to late-pregnancy needs to be further investigated.

Hastening postpartum involution

In mares, first postpartum estrus occurs within 5 - 20 days[53,54] while the uterus is still undergoing repair from tissue alterations during pregnancy and foaling. Uterine involution is not complete until 15 days postpartum and mares ovulating > 10 days postpartum are more likely to achieve and maintain pregnancy compared to those that ovulated earlier.[55] This is attributed to the interval of time (5.5 days) that the embryo resides within the oviduct after fertilization before migrating to uterine lumen, at which point involution should be complete. Therefore, progestins were used[56-58] in an attempt to delay ovulation in order to improve fertility at first postpartum estrus, with mixed results. One study found altrenogest to delay ovulation and increased pregnancy rates in postpartum mares when administered for the initial 8 days.[56] Additionally, altrenogest had no effect on other aspects of uterine involution, including uterine tone, size, or retention of fluid. A second study found altrenogest treatment to delay first ovulation by 10 days but did not improve pregnancy rates when fed for 15 days postpartum.[57]

Progesterone (100 mg) in oil treatment from days 5 to 14 postpartum successfully inhibited ovulation in 6/9 mares.[58] Additionally, the researchers noted no histological changes in treated mares, and pregnancy was achieved in 66% of treated mares, comparable to other estrous cycles. Addition of estradiol-17_{β} to this treatment was tried.[59] There was no delay in ovulation when mares were treated with either 150 mg progesterone in oil + 10 mg estradiol-17_{β} or 300 mg progesterone in oil + 20 mg estradiol-17_{β}, and pregnancy rates were comparable among the groups and controls.[59] It was noted that none of the P&E treated mares ovulated prior to 10 days postpartum, whereas 20% of control mares ovulated early. Additionally, less variability from time to ovulation was noted in treated mares. Overall, progestin treatment appears to successfully delay ovulation postpartum; however, there are conflicting data on its ability to improve first estrous cycle pregnancy rates.

Potential side effects of progestins to immune system

Progestins affect the immune system in a variety of species.[60] In humans, similar synthetic progestins to those used in mares are available for contraception, many of which have detrimental effect on the immune system. In most species, progesterone is highly antiinflammatory and enacts this function on the immune system through signaling on the PR.[61-64] In contrast, synthetic progestins such as MPA and levonorgestrel (LNG) increase the expression or production of a variety of proinflammatory mediators.[60,65-67] Additionally, the specific function of the individual progestin appears to be affected by the receptor through which it enacts, as MPA activates both PR and glucocorticoid receptors, whereas LNG solely activates the PR.[68,69] Through this increase in proinflammatory cytokine activation, in addition to recruitment of varying immune cells, women on synthetic progestins were found to be at higher risk for a variety of diseases, including HIV,[70-75] HSV-2,[76-78] chlamydia,[79,80] gonorrhea,[81] and even influenza.[60,82] It is believed that the increased risk of viral infection is due to proinflammatory activation of NFκβ that binds to the terminal repeat of viral DNA, thereby promoting viral replication.

A recent study investigated effects of altrenogest on the immune system of nonpregnant mares, with similar results to those reported in humans.[83] In vitro, altrenogest caused a dose-dependent

increase in the expression of the proinflammatory cytokine IL-1β in addition to the pleiotropic cytokine IL-6, in peripheral blood mononuclear cells. This was also noted in vivo, wherein under the diestrus influence of endogenous progesterone, control mares had decreased expression of proinflammatory cytokines (IFNγ, IL-1β, and IL-8) while the peripheral blood mononuclear cells of altrenogest-treated mares did not decrease in the expression of either IL-1β or IL-8. Additionally, altrenogest altered expression of a variety of cytokines within the endometrium, including IFNγ, IL-10, and IL-1RN, indicating both local and systemic effects of altrenogest on the immune system of the mare. This may be clinically relevant, as altrenogest treatment in nonpregnant mares has been associated with vaginal candidiasis[84] and uterine inflammation.[85] Interestingly, it was determined that altrenogest alters the immune system of the nonpregnant mare without activating glucocorticoid receptors. As it is structurally a 19-nortestosterone, and more comparable to LNG, this was not surprising.

In early pregnancy, altrenogest treatment decreased endometrial expression of the PR.[86] Additionally, an increase in the number of polymorphonuclear neutrophils was noted within the endometrium after treatment. In late pregnancy, minimal changes were observed in endometrial gene expression of IL-6, IL-8, or COX-2 after altrenogest treatment.[85] In this same study, altrenogest treatment had no effect on the expression of the progesterone, estrogen, or oxytocin receptors, conflicting with early pregnant mare data. This is interesting, as progesterone itself inhibited the NFκβ pathway, including downstream COX-2 and IL-1β,[87] again indicating a differing pathway of effect for altrenogest. Foals born to mares that were treated with altrenogest during late pregnancy up to the day of foaling were affected by treatment. This included an imbalanced neutrophil to lymphocyte ratio, increased cortisol, decreased potassium, and decreased calcium within hours after birth.[88] In an additional study, neonates born to mares receiving late-term altrenogest, had decreased respiratory function and substantially more problems in the perinatal period.[48]

Conclusion

Progestins are valuable clinical tools in managing several aspects of equine reproduction. Although only natural and synthetic (altrenogest) progestins are currently available, both compounds are efficacious in suppressing estrus, hastening transition, and maintaining early pregnancy. In the past, progestin treatment has been considered to be without side effects and perhaps overused as an insurance policy for pregnancy maintenance. Based on recent studies, long-term use of altrenogest, particularly during late pregnancy, may be associated with immune-related side effects in mares and foals. This possibility should be considered when benefits are weighed against risks in clinical situations (Figure 3).

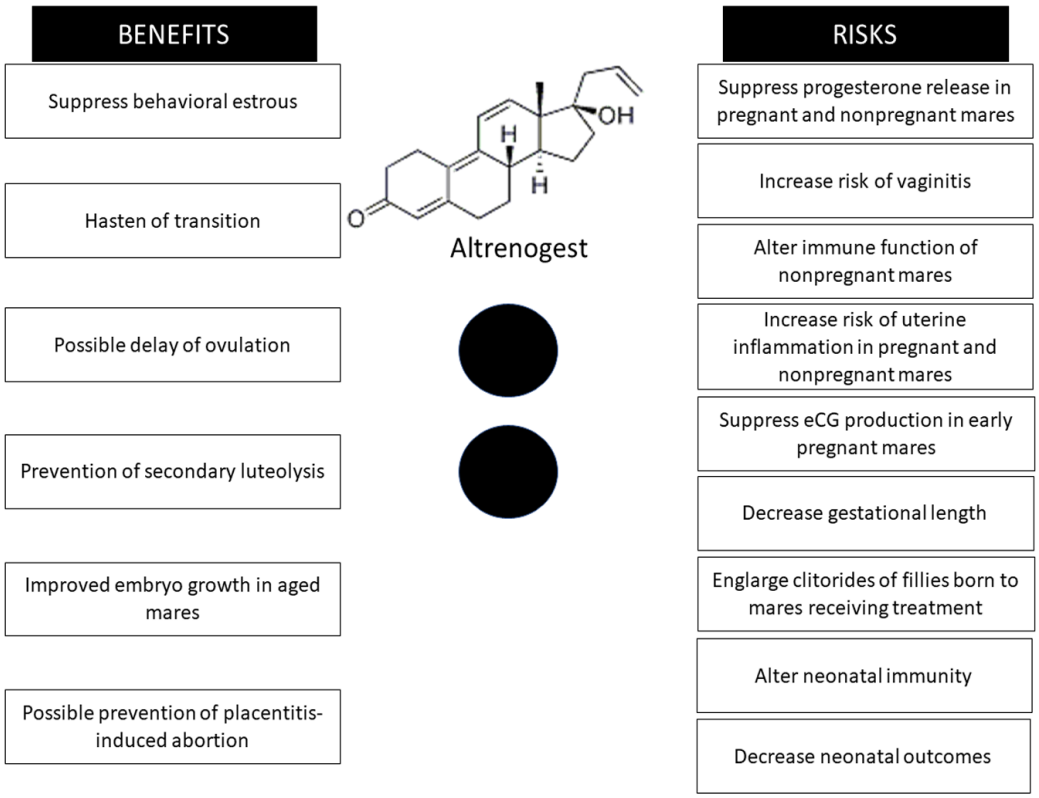

Figure 3. Risk to benefit ratio of altrenogest treatment

Conflict of interest

Author has no affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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