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REVIEW

Recent advances in the development of transdermal delivery systems for treatment of infertility

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Abstract: In vitro fertilization (IVF) has been utilized since the late 1970s. Since the time of the first successful pregnancy, use of IVF medication has changed over the years in regards to potency, timing, and types of medications given. Most commonly, these medications are given using a hypodermic needle, which causes some discomfort to the patient. Transdermal medications have several benefits, and this route could someday be utilized more frequently in IVF cycles. In this review, the current uses of transdermal medications in IVF are discussed, as well as future potential therapies.

Keywords: in vitro fertilization, transdermal, estradiol, testosterone

Introduction

Infertility is a disease that affects 15% of all couples. While various treatments have been offered for hundreds and even thousands of years, the field of reproductive endocrinology and infertility has expanded rapidly in the last four decades with the advent of in vitro fertilization (IVF), intracytoplasmic sperm injection, ovulation induction agents, and the discovery and pharmaceutical production of gonadotropin-releasing hormone (GnRH) analogs and exogenous gonadotropins. Since the initial isolation of sex steroids and the first live IVF birth, physicians and scientists have been experimenting with GnRH analogs, gonadotropins, and sex steroids to make them more efficient and improve fertility outcomes. Initially, different chemical formulations and doses were pursued for maximum potency and efficiency. This was followed by exploration of different routes for administration of fertility drugs to include intramuscular, subcutaneous, oral, nasal, vaginal, and transdermal. As infertility treatments have evolved, different combinations of medications and protocols have been utilized for induction of ovulation, controlled ovarian stimulation, and luteal phase support. This has included the use of transdermal medications. As infertility treatment utilization and success rates have increased, there is an increasing demand for safer, less invasive, and more cost-effective treatments.

Transdermal drugs represent an attractive alternative to oral medications and are especially appealing when compared with hypodermic injection. Human civilizations have been applying medicinal agents to the skin for thousands of years.[1] However, it was not until very recently that the skin became a targeted drug route. There are several advantages to transdermal therapy. Foremost, it is useful when there is a significant first-pass effect of the liver that prematurely metabolizes the drug. The transdermal route also has advantages over the hypodermic needle, which can be painful and generate

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dangerous medical waste. Other advantages include its noninvasive nature and ease of self-administration.[2] During controlled ovarian hyperstimulation, gonadotropins are given for about 2 weeks. Transdermal drugs could be used for this therapy as they provide release of drugs for long periods of time, and in the case of transdermal contraceptive use, they increase compliance.[3]

While transdermal drug administration has advantages that make it an attractive route, there are several limitations that have restricted the number of drugs that can be used via this delivery system. Drug size must be a few hundred Daltons or less, lipophilic, and require doses in milligrams per day or less.[1] These restraints have posed challenges over the years; however, several techniques have been employed to circumvent these obstacles. These techniques can be broken down into different generations of transdermal medications.

First-generation patches are considered the classic patches that were made initially. Estradiol was the first hormone to be converted to a transdermal medication. First-generation medications must have a low molecular weight, be lipophilic, and be efficacious at low doses. In almost all patch designs, a drug reservoir is encased by an impermeable backing and an adhesive that is in contact with the skin.[4] Second-generation transdermal delivery recognizes the challenge of larger molecules penetrating the skin and attempts to circumvent this by reversibly disrupting the stratum corneum, provide an added driving force and avoiding injury to deeper tissues. This is particularly difficult given that disruption of the stratum corneum often causes some skin irritation. Third-generation transdermal therapy focuses more intensely on the stratum corneum. This generation of transdermal drugs has expanded to the use of microneedles and microdermabrasion to allow macromolecules to cross the skin barrier.[5] Currently, transdermal drugs used for IVF consist of estrogen and testosterone.

However, the advancing technology is particularly exciting as it may provide opportunities for traditional therapies to be administered via new routes and may alter the way in which certain infertility states are treated.

Despite these potential benefits, transdermal medication for infertility currently has a limited role in clinical medicine. This is the direct result of very few hormones with ability to cross the stratum corneum. Testosterone and estrogen are the only medications approved by the US Food and Drug Administration that have the ability to be given via the transdermal route. Gonadotropins like follicle-stimulating hormone (FSH) and luteinizing hormone have a high molecular weight and would need a second-generation or third-generation drug delivery system.

Literature search

A database search was performed in PubMed on September 10, 2014 using the key words "transdermal medications", "infertility", and "in vitro fertilization". The search included articles from 2000 to 2014 and was performed using MEDLINE. Any article discussing transdermal medications used in IVF was included. This search yielded 24 full text papers, which were evaluated. The search terms utilized were (transdermal[tiab] OR cutaneous[tiab] OR administration, cutaneous[mh] OR dermal[tiab] OR transcutaneous[tiab] OR (skin[tiab] AND (administration[tiab] OR administer[tiab])) AND (fertility agents[mh] OR fertility agents [pharmacological action] OR buserelin[mh] OR cetrorelix[sc] OR clomiphene[mh] OR enclomiphene[mh] OR gonadotropins, pituitary[mh] OR leuprolide[mh] OR menotropins[mh] OR nafarelin[mh] OR onapristone[sc] OR zuclomiphene[mh] OR androgens[mh] OR androgens[pharmacologic action] OR testosterone[mh] OR testosterone[tiab] OR estradiol[mh] OR estradiol[tiab] OR estrogens[pharmacological action] OR estrogens[mh]) AND (infertil*[tiab] OR fertil*[tiab] OR IVF[tiab] OR in-vitro fertiliz* OR fertilization in vitro[mh] OR menopause, premature[mh] OR primary ovarian insufficiency[mh] OR reproductive techniques, assisted[mh] OR infertility[mh] OR sterility[tiab]). Articles were reviewed by one author (MC) and selected articles reviewed by a second author (MJH). Additional articles of interest were identified from references cited in these papers. All articles evaluating transdermal fertility medications were included and no additional exclusion criteria were applied. The current review summarizes the transdermal medications currently used in IVF.

Transdermal estrogen for preparation of the endometrium

Implantation is dependent upon endometrial receptivity and developmental synchrony with the developing embryo, and these events are mediated by the action of estrogen and progesterone.[6] The action of estrogen in the follicular phase is mitogenic to endometrial cells, causing rapid stromal and glandular cell growth. With rising progesterone after ovulation, estrogen activity in the luteal endometrium is inhibited and there is a decline in mitotic activity and DNA synthesis. The spiral vessels become more tortuous and the stroma becomes edematous, with upregulation of key implantation factors. In naturally conceiving cycles, these

events are exquisitely timed such that release of progesterone is timed to coincide with ovulation, so that endometrial changes are precisely coordinated with growth of the embryo. The embryo leaves the fallopian tube as a blastocyst and enters the endometrial cavity, while the endometrium enters a brief window of embryo receptivity. However, in patients who undergo fertility therapy, iatrogenic asynchrony between the embryo and endometrium often develops. This is especially true for patients who utilize donor oocytes or undergo transfer of frozen embryos, where GnRH analogs are used to prevent ovulation. This results in a lack of production of estrogen in the ovaries. In these circumstances, a natural cycle may be used. However, this requires intensive monitoring with transvaginal ultrasound to ensure follicular development and ovulation. Once development is noted, testing for urinary luteinizing hormone and further ultrasound are needed to confirm the timing of ovulation. Ovulation predictor kits can be difficult for patients to handle and cancellation rates may be high.[7] Furthermore, development of the endometrium in the follicular phase is affected by age, and may be a contributing factor to lower pregnancy rates in older women.[8] Given the difficulties in timing frozen embryos or donor embryos in a natural cycle, an alternative to this intensive regimen may be sequential exogenous estrogen and progesterone to induce endometrial receptivity. Less frequent ultrasound examinations are required and the length of the follicular phase can be varied without negatively affecting pregnancy rates.[9,10]

Estrogen may be given orally, transdermally, or vaginally. Orally administered estradiol valerate must pass through the intestine, where some of the drug is converted to estrone.[11] Thereafter, the estradiol is further metabolized to estrone and estriol in the liver. Approximately 30% of the estradiol is metabolized into forms that are inactive at the target organ system.[12] Transdermal estrogen avoids the intestinal milieu and is not metabolized by the liver. Other benefits of the transdermal route include the avoidance of increasing lipoproteins, clotting factors, and the renin substrate.[13] Vaginal administration of estrogen also avoids metabolic effects, but can cause local irritation and discomfort. These side effects may decrease compliance. Furthermore, it has been shown that vaginal estrogen may inhibit the absorption of vaginal progesterone, which is needed for development of the endometrium.[14]

Transdermal estrogen is typically given as 0.2–0.4 mg daily.[10,15] This regimen is designed to achieve serum estrogen levels approximating those in the late follicular phase. The duration of estrogen therapy can vary from as little as 7 days to more than 30 days, and can involve sequential increases to up to four patches being applied per day.[10,15] The lining of the endometrium is monitored with transvaginal ultrasound and the goal is to achieve a lining that is greater than 6–7 mm in thickness.[16,17] A randomized controlled trial comparing oral versus transdermal estrogen for frozen embryo transfers found no difference in pregnancy outcomes between the two administration routes.[18] Several other studies have reported good pregnancy outcomes with transdermal estrogen administration for frozen-thawed embryo and donor embryo transfers.[19–22] Transdermal estrogen for preparation of the endometrial lining in frozen-thawed embryo and donor embryo transfer cycles appears to be effective and has outcomes similar to those when using other routes of estrogen administration.

Transdermal estrogen for luteal phase support

During the early years of IVF, there was a report indicating that estradiol levels dramatically decreased at the end of the luteal phase.[23] This prompted questions as to whether or not estrogen replacement was needed during the luteal phase for successful outcomes. Several trials have attempted to establish the benefit of addition of estradiol (oral or transdermal) to progesterone for luteal support after fresh autologous IVF cycles. Three meta-analyses have been performed, and concluded that adding estrogen for luteal support is of no benefit.[24–26] This is consistent with the results of a recent Cochrane review showing no benefit of luteal estrogen.[27]

However, the studies summarized in these meta-analysis utilized human chorionic gonadotropin (hCG) triggers to induce final oocyte maturation. The long half-life of hCG compared with that of luteinizing hormone results in continued stimulation of the corpus luteum after oocyte retrieval, leading to sustained release of endogenous estrogen and progesterone.[28] Recently, GnRH agonists have been used to trigger release of endogenous luteinizing hormone, resulting in oocyte maturation followed by rapid involution of the corpus luteum and a decreased risk of ovarian hyperstimulation syndrome. However, this results in a dysfunctional luteal hormonal milieu with low levels of estrogen and progesterone.[29,30] Clinicians have implemented intensive luteal phase support protocols after GnRH agonist triggering to replace the deficient corpus luteum hormone production. This can only be accomplished by using a combination of intramuscular progesterone and either oral or transdermal estrogen. Engmann et al utilized 0.1 mg transdermal estradiol patches in a randomized controlled trial comparing hCG with GnRH

agonist triggers.[31] The patients applied three estradiol patches every other day to support the luteal phase in the GnRH agonist arm. With this intensive luteal phase support, GnRH agonist triggers achieved a similar ongoing pregnancy rate of 53% compared with 48% in the hCG trigger arm. Thus, transdermal estrogen appears to be an effective component of the intensive luteal support protocol following GnRH agonist triggering.

Follicular synchronization using transdermal estrogen

Some patients undergoing IVF are "poor responders". While various definitions have been used, these patients tend to have fewer mature follicles and lower peak estradiol values, and have higher rates of canceled cycles secondary to minimal response. While age and ovarian reserve play a role in poor response, it is not always clear why patients respond poorly to gonadotropin stimulation. These patients often display asynchronous follicular development, which may partly explain their poor response to stimulation.[32] This particular subset of patient represent a difficult challenge for clinicians. Despite strategies to optimize responses, these patients suffer from high IVF cancellation rates.[33] Protocols derived to optimize outcome in this subset of patients focuses on maximizing the number of responsive follicles.

Patients with diminished ovarian reserve have lower levels of inhibin B due to fewer ovarian follicles. This leads to higher and earlier release of FSH from the pituitary, resulting in early recruitment of some follicles. This can negative impact IVF cycles in these patients, due to asynchronous recruitment of the available follicular pool. In order to optimize follicular recruitment, a luteal phase GnRH antagonist can be given as well as estradiol (typically 0.3 mg patches) in order to downregulate FSH. Neither GnRH nor estradiol has been shown to be superior. In terms of cost, transdermal estradiol is less expensive and may be preferred for this reason. This approach improves follicular synchrony for follicular phase stimulation and decreases the heterogeneity of follicular size.[34] In one report, patients undergoing this regimen with the estradiol patch had fewer cancellations, more oocytes retrieved, and more embryos transferred when compared with their prior cycles.[33] Furthermore, a meta-analysis looking at GnRH antagonist cycles with and without estradiol pretreatment found that there were fewer cancellations and a higher chance of clinical pregnancy with luteal estradiol pretreatment.[35] Estradiol patch luteal priming may be a method to improve IVF cycle outcomes in poor responders, but there is a need for more randomized controlled trials to address this question.

Transdermal progesterone

While estrogen replacement is important in several specific scenarios for infertility, exogenous progesterone support in the luteal phase plays a much larger role. This includes fresh IVF, frozen-thawed embryo transfers, donor oocyte transfers, and induction of ovulation with gonadotropins.[36] Due to its ubiquitous use in infertility treatment, progesterone would be an ideal option for transdermal delivery. However, when progesterone is administered transdermally, it is rapidly metabolized by 5α-reductase to 5α-dihydroprogesterone, which lowers plasma progesterone levels. This rapid metabolism makes transdermal progesterone less than ideal. Thus, transdermal progesterone patches are not commercially available for use in IVF. However, transdermal progestins are available in patch formulations for contraception and hormone replacement. For example, transdermal norelgestromin is metabolized to levonorgestrel for contraceptive effect. While progesterone creams are available, they are not regulated by the US Food and Drug Administration and have considerable variation in the amount and concentration of progesterone delivered. In current forms, progesterone is not able to be given transdermally for IVF. Currently, progesterone is administered via the intramuscular or vaginal route for fertility treatment.

Transdermal testosterone for poor responders

The two-cell two-gonadotropin theory posits that androgens play a crucial role in the synthesis of steroids. The aromatase activity in granulosa cells converts androgens to estrogens.[37] Furthermore, androgens have been shown to exert a paracrine effect on ovarian physiology and upregulate FSH receptor expression during follicular growth.[38–40] Further evidence pointing to the importance of androgens during IVF is the positive correlation between serum testosterone and number of oocytes retrieved, as well as the higher pregnancy rates seen in patients with basal testosterone levels above 20 ng/dL.[41] Poor responders tend to be slightly older in age, and Barbieri et al showed that serum testosterone decreases significantly with age in women undergoing IVF.[42] Dehydroepiandrosterone is responsible for almost half of the testosterone in follicular fluid during controlled ovarian hyperstimulation.[43] This knowledge had prompted the question of testosterone priming for poor responders. Several small non-randomized

controlled trials of oral dehydroepiandrosterone showed an increase in mature oocytes and pregnancy rates.[44–46] Better quality evidence exists for the use of transdermal testosterone. A recent meta-analysis consisting of a total of 221 patients who were involved in three randomized controlled trials showed higher clinical pregnancy rates, a higher live birth rate, and lower amounts of FSH utilized in the testosterone group. However, there was no difference in number of oocytes used and cancellation rates did not differ. The regimens used were a transdermal testosterone gel and a 2.5 mg testosterone patch.[47] While the numbers are relatively small, there is some promise in using this regimen.

Future challenges

The current body of literature on transdermal medication is relatively small. However, there is evidence that certain protocols utilizing transdermal medications are useful. This is especially true for donor oocyte and frozen embryo transfer protocols. While evidence exists for poor responders, larger studies are needed to confirm the effect of testosterone in poor responders. Transdermal medication use for IVF is relatively small as only two hormones are available for transdermal use at present. As mentioned earlier, these are first-generation patches. Other hormones (FSH, luteinizing hormone, GnRH, hCG, and progesterone) currently used are not able to utilize a first-generation transdermal patch. With advances in transdermal medication, this may change in the coming years. Recently, the first pregnancy using a microporation laser and transdermal FSH patch was described.[48] This technology has the potential to revolutionize IVF therapy. However, further studies are needed to determine if this is a viable therapy for patients. In addition, companies are actively looking to develop transdermal GnRH patches.[49] The current patent for transdermal GnRH would utilize an iontophoresis system. This would have the potential to dramatically change the treatment for certain conditions. Patients with no endogenous GnRH could avoid injectable medications for controlled ovarian stimulation and potentially utilize this therapy for development of secondary sexual characteristics when entering puberty. While there are numerous technical challenges in developing transdermal applications for new fertility medications, there is a large opportunity for this route of administration.

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Disclosure

The authors report no conflicts of interest in this work. The views expressed in this manuscript are those of the authors and do not reflect the official policy or position of the Department of Health and Human Services, Department of Defense, or the US government.

References

1. Prausnitz MR, Langer R. Transdermal drug delivery. *Nat Biotechnol*. 2008;26(11):1261–1268.
2. Dedakia A, Matholiya C, Koyani V, Bhimani D. Three generations: primary, secondary, and tertiary generations of transdermal drug delivery systems: a review. *Int J Pharm Sci Res*. 2013;4(6):2159–2173.
3. Audet MC, Moreau M, Koltun WD, et al. Evaluation of contraceptive efficacy and cycle control of a transdermal contraceptive patch vs an oral contraceptive: a randomized controlled trial. *JAMA*. 2001;285(18):2347–2354.
4. Venkatraman S, Gale R. Skin adhesives and skin adhesion. 1. Transdermal drug delivery systems. *Biomaterials*. 1998;19(13):1119–1136.
5. Arora A, Prausnitz MR, Mitragotri S. Micro-scale devices for transdermal drug delivery. *Int J Pharm*. 2008;364(2):227–236.
6. Cheong Y, Boomsma C, Heijnen C, Macklon N. Uterine secretomics: a window on the maternal-embryo interface. *Fertil Steril*. 2013;99(4):1093–1099.
7. Sathanandan M, Macnamee MC, Rainsbury P, Wick K, Brinsden P, Edwards RG. Replacement of frozen-thawed embryos in artificial and natural cycles: a prospective semi-randomized study. *Hum Reprod*. 1991;6(5):685–687.
8. Sher G, Herbert C, Maassarani G, Jacobs MH. Assessment of the late proliferative phase endometrium by ultrasonography in patients undergoing in-vitro fertilization and embryo transfer (IVF/ET). *Hum Reprod*. 1991;6(2):232–237.
9. Leeton J, Rogers P, Healy D. A comparison of pregnancy rates for 131 donor oocyte transfers using either a sequential or fixed regime of steroid replacement therapy. *Hum Reprod*. 1991;6(2):299–301.
10. Navot D, Anderson T, Droesch K, Scott R, Kreiner D, Rosenwaks Z. Hormonal manipulation of endometrial maturation. *J Clin Endocrinol Metab*. 1989;68(4):801–807.
11. Ryan KJ, Engel LL. The interconversion of estrone and estradiol by human tissue slices. *Endocrinology*. 1953;52(3):287–291.
12. Campbell S, Whitehead M. Potency and hepatocellular effects of oestrogens after oral, percutaneous and subcutaneous administration. In: Van Keep P, Utain W, Vermeulen A, editors. *The Controversial Climacteric*. Lancaster, UK: MTP Press; 1982.
13. Powers M, Schenkel L, Darby PE, Good WR, Balestra JC, Place VA. Pharmacokinetics and pharmacodynamics of transdermal dosage forms of 17 beta-estradiol: comparison with conventional oral estrogens used for hormone replacement. *Am J Obstet Gynecol*. 1985;152(8):1099–1106.
14. Rosenwaks Z. Donor eggs: their application in modern reproductive technologies. *Fertil Steril*. 1987;47(6):895–909.
15. Hancke K, More S, Kreienberg R, Jurge W. Patients undergoing frozen-thawed embryo transfer have similar live birth rates in spontaneous and artificial cycles. *J Assist Reprod Genet*. 2012;29(5):403–407.
16. Gonen Y, Casper RF, Jacobson W, Blankier J. Endometrial thickness and growth during ovarian stimulation: a possible predictor of implantation in in vitro fertilization. *Fertil Steril*. 1989;52(3):446–450.

17. Bourgain C, Devroey P, Van Waesberghe L, Smitz J, Van Seirteghem AC. Effects of natural progesterone on the morphology of the endometrium in patients with primary ovarian failure. *Hum Reprod*. 1990;5(5):537–543.
18. Janati S, Davar R, Mohseni F. Comparison of effect of transdermal estradiol and estradiol valerate on endometrial receptivity in frozen-thawed embryo transfer cycles. *Iran J Reprod Med*. 2013; 11(4 Suppl):S68.
19. Dal Prato L, Borini A, Cattoli M, Bonu MA, Sciajno R, Flamigni C. Endometrial preparation for frozen-thawed embryo transfer with or without pretreatment with gonadotropin-releasing hormone agonist. *Fertil Steril*. 2002;77(5):956–960.
20. Bals-Pratsch M, Al-Hasani S, Schopper B, et al. A simple, inexpensive and effective artificial cycle with exogenous transdermal oestradiol and vaginal progesterone for the transfer of cryopreserved pronucleated human oocytes in women with normal cycles. *Hum Reprod*. 1999; 14 Suppl 1:222–230.
21. Banz C, Katalinic A, Al-Hasani S, et al. Preparation of cycles for cryopreservation transfers using estradiol patches and Crinone 8% vaginal gel is effective and does not need monitoring. *Eur J Obstet Gynecol Reprod Biol*. 2002;103(1):43–47.
22. Devroey P, Pados G. Preparation of endometrium for egg donatin. *Hum Reprod Update*. 1998;4(6):856–861.
23. Smitz J, Devroey P, Braeckmans P, et al. Management of failed cycles in an IVF/GIFT programme with the combination of a GnRH analogue and HMG. *Hum Reprod*. 1987;2(4):309–314.
24. Kolibianakis EM, Venetis CA, Papanikolaou EG, Diedrich K, Tarlatzis BC, Griesinger G. Estrogen addition to progesterone for luteal phase support in cycles stimulated with GnRH analogues and gonadorophins for IVF: a systematic review and meta-analysis. *Hum Reprod*. 2008;23(6):146–154.
25. Gelbaya TA, Kyrgiou M, Tsoumpou I, Nardo LG. The use of estradiol for luteal phase support in in vitro fertilization/intracytoplasmic sperm injection cycles: a systematic review and meta-analysis. *Fertil Steril*. 2008;90(6):2116–2125.
26. Jee BC, Suh CS, Kim SH, Kim YB, Moon SY. Effects of estradiol supplementation during the luteal phase of in vitro fertilization cycles: a meta-analysis. *Fertil Steril*. 2010;92(2):428–436.
27. van der Linden M, Buckingham K, Farquhar C, Kremer JA, Metwally M. Luteal phase support for assisted reproduction cycles. *Cochrane Database Syst Rev*. 2011;10:CD009154.
28. Connell MT, Szatkowski JM, Terry N, DeCherney AH, Propst AM, Hill MJ. Timing luteal support in assisted reproductive technology: a systematic review. *Fertil Steril*. 2015;103(4):939–946.
29. Humaidan P, Engmann L, Benadiva C. Luteal phase supplementation after gonadotropin-releasing hormone agonise trigger in fresh embryo transfer: the American versus European approaches. *Fertil Steril*. 2015;103(4):879–885.
30. Beckers NG, Macklon NS, Eijkemans MJ, et al. Nonsupplemented luteal phase characteristics after the administration of recombinant human chorionic gonadotropin, recombinant luteinizing hormone, or gonadotropin-releasing hormone (GnRH)agonist to induce final oocyte maturation in in vitro fertilization patients after ovarian stimulation with recombinant follicle-stimulating hormone and GnRH antagonist cotreatment. *J Clin Endocrinol Metab*. 2003;88(9): 4186–4192.
31. Engmann L, DiLuigi A, Schmidt D, Nulsen J, Maier D, Benadiva C. The use of gonadotropin-releasing hormone (GnRH) agonist to induce oocyte maturation after cotreatment with GnRH antagonist in high-risk patients undergoing in vitro fertilization prevents the risk of ovarian hyperstimulation syndrome: a prospective randomized controlled study. *Fertil Steril*. 2008;89(1):84–91.
32. Humaidan P, Bungum L, Bungum M, et al. Reproductive outcome using a GnRH antagonist (cetrorelix) for luteolysis and follicular synchronization in poor responder IVF/ICSI patients treated with a flexible GnRH antagonist protocol. *Reprod Biomed Online*. 2005;11(6):679–684.
33. Dragisic K, Davis O, Fasouliotis S, Rosenwaks Z. Use of a luteal estradiol patch and a gonadotropin-releasing hormone antagonist suppression protocol before gonadotropin stimulation for in vitro fertilization in poor responders. *Fertil Steril*. 2005;84(4):1023–1026.
34. Fanchin R, Salomon L, Castelo-Branco A, Olivennes F, Frydman N, Frydman R. Luteal estradiol pre-treatment coordinates follicular growth during controlled ovarian hyperstimulation with GnRH antagonists. *Hum Reprod*. 2003;18(12):2698–2703.
35. Reynolds KA, Omurtag KR, Jimenez PT, Rhee JS, Tuuli MG, Jungheim ES. Cycle cancellation and pregnancy after luteal estradiol priming in women defined as poor responders: a systematic review and meta-analysis. *Hum Reprod*. 2013;28(11):2981–2989.
36. Hill MJ, Whitcomb BW, Lewis TD, et al. Progesterone luteal support after ovulation induction and intrauterine insemination: a systematic review and meta-analysis. *Fertil Steril*. 2013;100(5):1373–1380.
37. Ryan KJ, Petro Z, Kaiser J. Steroid formation by isolated and recombined ovarian granulosa and theca cell. *J Clin Endocrinol Metab*. 1968;28(3):355–358.
38. Weil SJ, Vendola K, Zhou J, et al. Androgen receptor gene expression in the primate ovary: cellular localization, regulation, and functional correlations. *J Clin Endocrinol Metab*. 1998;83(7):2479–2485.
39. Vendola KA, Zhou J, Adesanya OO, Weil SJ, Bondy CA. Androgens stimulate early stages of follicular growth in the primate ovary. *J Clin Invest*. 1998;101(12):2622–2629.
40. Vendola K, Zhou J, Wang J, Famuyiwa OA, Bievre M, Bondy CA. Androgens promote oocyte insulin-like growth factor I expression and initiation of follicle development in the primate ovary. *Biol Reprod*. 1999;61(2):353–357.
41. Frattarelli JL, Peterson EH. Effect of androgen levels on in vitro fertilization cycles. *Fertil Steril*. 2004;81(6):1713–1714.
42. Barbieri RL, Sluss PM, Powers RD, et al. Association of body mass index, age, and cigarette smoking with serum testosterone levels in cycling women undergoing in vitro fertilization. *Fertil Steril*. 2005; 83(2):302–308.
43. Haning RV Jr, Hackett RJ, Flood CA, Loughlin JS, Zhao QY, Longcope C. Plasma dehydroepiandrosterone sulfate serves as a prehormone for 48% of follicular fluid testosterone during treatment with menotropins. *J Clin Endocrinol Metab*. 1993;76(5):1301–1307.
44. Artini PG, Simi G, Ruggiero M, et al. DHEA supplementation improves follicular microenviroment in poor responder patients. *Gynecol Endocrinol*. 2012;28(9):669–673.
45. Sonmezer M, Ozmen B, Cil AP, et al. Dehydroepiandrosterone supplementation improves ovarian response and cycle outcome in poor responders. *Reprod Biomed Online*. 2009;19(4):508–513.
46. Barad D, Brill H, Gleicher N. Update on the use of dehydroepiandrosterone supplementation among women with diminished ovarian function. *J Assist Reprod Genet*. 2007;24(12):629–634.
47. Luo S, Li S, Li X, Qin L, Jin S. Effect of pretreatment with transdermal testosterone on poor ovarian responders undergoing IVF/ICSI: a meta-analysis. *Exp Ther Med*. 2014;8(1):187–194.
48. Zech NH, Murtinger M, Uher P. Pregnancy after ovarian superovulation by transdermal delivery of follicle-stimulating hormone. *Fertil Steril*. 2011;95(8):2784–2785.
49. NIH Small Business Innovation Research, Small Business Technology Transfer Programs. Transdermal GnRH delivery system to treat infertility. Bethesda, MD, USA: National Institutes of Health; Available from: https://www.sbir.gov/sbirsearch/detail/77730. Accessed February 16, 2015.