



Research article

Dynamic changes in serum steroid hormone during the first trimester of pregnancy between infertile women conceiving with and without hormone replacement therapy

Ayumu Ito ^{a,b,c}, Yukiko Katagiri ^{a,b,c,*}, Yusuke Fukuda ^{b,c}, Mineto Morita ^{a,b}^a Department of Obstetrics and Gynecology, Toho University Graduate School of Medicine, 5-21-16 Omorinishi Ota-ku, Tokyo, Japan^b Department of Obstetrics and Gynecology, Faculty of Medicine, Toho University, 5-21-16 Omorinishi Ota-ku, Tokyo, Japan^c Reproduction Center, Toho University Omori Medical Center, 6-11-1 Omorinishi Ota-ku, Tokyo, Japan

ARTICLE INFO

Keywords:

Breast cancer
Hormone receptor-positive
Cancer survivor
Oncofertility
Fertility preservation
Adolescent and young adult
HRT
Frozen-thawed embryo transfer
Estrogen
Progesterone

ABSTRACT

Objective: Recent advances in cancer treatment and reproductive medicine have made the post-treatment quality of life an important concern for cancer survivors. We aimed to evaluate the safety of sex hormone (estradiol and progesterone) replacement therapy (HRT) in women who conceived by assisted reproductive technology (ART) with hormone receptor-positive breast cancer.**Methods:** We measured serum E2 and P4 levels at 4–10 weeks of gestation in women who conceived naturally or after timed intercourse or intrauterine insemination for infertility without HRT for luteal support (non-HR group; n = 135). We conducted a retrospective comparison of the values from the non-HR group with those of women who conceived by ART with HRT for infertility (HR group; n = 75).**Results:** Serum E2 levels were significantly higher in the non-HR group than in the HR group at 5, 6, and 8 weeks of gestation. Similarly, serum P4 levels were significantly higher in the non-HR group than in the HR group at 4, 5, and 6 weeks of gestation.**Conclusions:** This study suggests that in cancer reproductive medicine for hormone-dependent breast cancer survivors, HRT administered during the first trimester of a pregnancy after primary disease treatment may not increase the sex hormone levels to levels above those seen in spontaneous pregnancy.

1. Introduction

Recent advancement in cancer treatment and reproductive medicine has increased the importance of post-treatment quality of life among childhood, adolescent, and young adulthood cancer survivors. Fertility preservation is of major concern, and the use of assisted reproductive technology (ART), such as cryopreservation of embryos, oocytes, or ovarian tissue, is important for conserving fertility. For women with a history of breast cancer and other hormone-sensitive malignancies, hormone replacement therapy (HRT), which is important for continued pregnancy using ART, risks the exacerbation or recurrence of the primary disease. Hormonal exposure owing to pregnancy could be also a risk factor; however, some studies have reported that the prognosis of breast cancer survivors who underwent appropriate neoadjuvant therapy is not necessarily worsened by spontaneous pregnancy [1, 2]. Although the rates of recurrence and mortality are

lower for patients who receive multidrug chemotherapy following breast cancer surgery than for those treated by surgery alone [3], patients who receive multidrug chemotherapy show decreased fertility owing to chemotherapy-induced ovarian failure and age-associated ovarian dysfunction resulting from prolonged administration of hormone therapy. Furthermore, many patients encounter difficulty in conceiving naturally. The levels of anti-Mullerian hormone (AMH), a parameter of ovarian reserve, decrease to undetectable levels during chemotherapy and remain low even after completing of chemotherapy [4]. Studies have reported low rates of pregnancy in breast cancer survivors [5, 6]. If ovarian function decreased and ovulation is impaired after cancer treatment, HRT is essential for embryo transfer using frozen embryos or oocytes once the patient is allowed to conceive. Even after pregnancy is established, this support must be continued until the main site of hormone production switches from the corpus luteum to the placenta (the luteo-placental shift).

* Corresponding author.

E-mail address: yukikonk@med.toho-u.ac.jp (Y. Katagiri).

In this study, we aimed to evaluate the safety of HRT in women who conceived by ART with hormone receptor-positive breast cancer.

2. Materials and methods

We measured the serum E2 and P4 levels at 4–10 weeks of gestation in non-HR group participants. The study subjects were women treated in the Department of Obstetrics and Gynecology or Reproduction Center, Toho University Omori Medical Center, between November 2018 and April 2019, who conceived naturally or after TI or IUI for infertility. The non-HR group participants did not undergo HRT for luteal support (Figure 1). TI and IUI cycles were performed naturally or using medication for ovulation induction with follicle growth monitoring by ultrasonography.

We retrospectively compared the serum E2 and P4 levels at 4–10 weeks of gestation between the non-HR and HR groups. The HR group included women who conceived after frozen-thawed embryo transfer and HRT with estrogen and progesterone in our reproduction center between January and December 2018 (Figure 1). The members of both groups continued their pregnancies until at least 12 weeks of gestation.

Serum E2 and P4 were measured by the Electrochemiluminescence immunoassay method using the cobas 8000 (Roche Diagnostics K.K., Tokyo, Japan). The assay reagents used were EClusys E2 IV (Roche Diagnostics K.K., Tokyo, Japan) for E2 and EClusys Progesterone III (Roche Diagnostics K.K., Tokyo, Japan) for P4.

Estrogen replacement therapy was administered in the form of transdermal estrogen patch (Estrana tape, Hisamitsu Pharmaceutical, Saga, Japan) applied every alternate day from day 3 of menstruation. The initial dose was 2.16 mg, and this was increased after a few days to 2.88 mg and then to 3.60 mg. Transvaginal natural progesterone (Lutinus Vaginal Tablets, Ferring Pharmaceutical, Tokyo, Japan) at a dose of 90–800 mg/day was administered when the thickness of the endometrium was ≥ 8 mm; depending on the stage of the frozen embryo to be transferred, the embryo transfer was performed on day 2 (P+2), day 3 (P+3), or day 5 (P+5) after the first day of administration of transvaginal natural progesterone use (P0). The serum hCG level was measured at 4w0d of gestation counted from the date of transfer. If the result was positive, HR was continued, and pregnancy was confirmed when the gestational sac was visible. The serum E2 and P4 levels were subsequently measured, and the administration of transdermal estrogen and

transvaginal natural progesterone was continued until adequate endogenous hormone secretion was confirmed after 7 weeks of gestation.

SPSS Statistics ver. 25 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. The difference between the data of non-HR group and HR group was tested via t-test. $P < 0.05$ is considered as the different significance.

2.1. Ethics declarations

The protocol of the study was approved by the Ethics Committee of Toho University Omori Medical Center (Approval No. M1704717209 and M18239) and conformed with the 1964 guidelines of the Helsinki Declaration and its later amendments. Informed consent was obtained from all patients. We obtained written informed consent from the patients of non-HR group and informed consent in the form of opt-out on the website from the patients of HR group. Information on the research was made public on the website of each institution, and the opportunity for the research subjects to refuse participation was guaranteed.

3. Results

The study population included 135 women who conceived naturally without the use of HRT (non-HR group); of these, 100 had conceived naturally and 35 had conceived after IUI) and 75 who conceived after frozen-thawed embryo transfer and HRT with estrogen and progesterone in our Reproduction Center between January and December 2018 (HR group). The age of the patients between the two groups were 33.4 ± 5.0 vs. 35.3 ± 4.2 years (non-HR group vs. HR group, $p = 0.012$) and the BMI were 21.7 ± 4.0 vs. 21.4 ± 2.9 kg/m² (non-HR group vs. HR group, $p = 0.496$).

The serum E2 and P4 levels in the non-HR and HR groups increased over time during the first trimester of pregnancy (Figures 2a, 2b, 3a, and 3b). There was no significant difference in the pregnancy continuation rate between the two groups.

The serum E2 level was significantly higher in the non-HR group than in the HR group at 5, 6, and 8 weeks of gestation (5 weeks, $p < 0.05$; 6 weeks, $p < 0.01$; 8 weeks, $p < 0.01$). There was no significant difference in the levels at 4, 7, 9, or 10 weeks of gestation between the groups (Table 1).

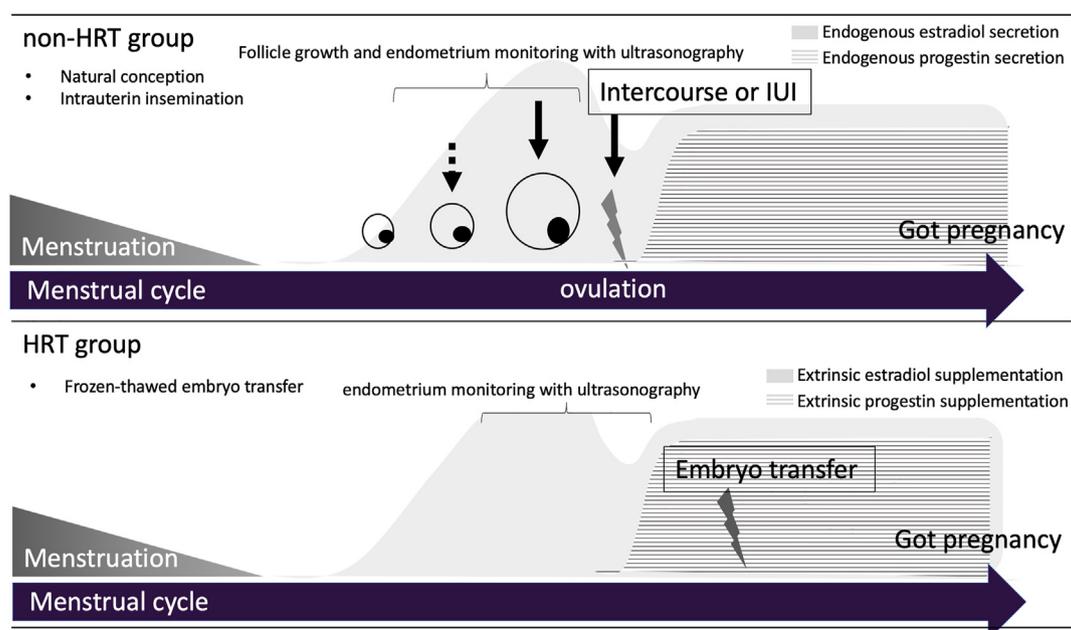


Figure 1. Medical treatment schedules of non-HR group and HR-group.

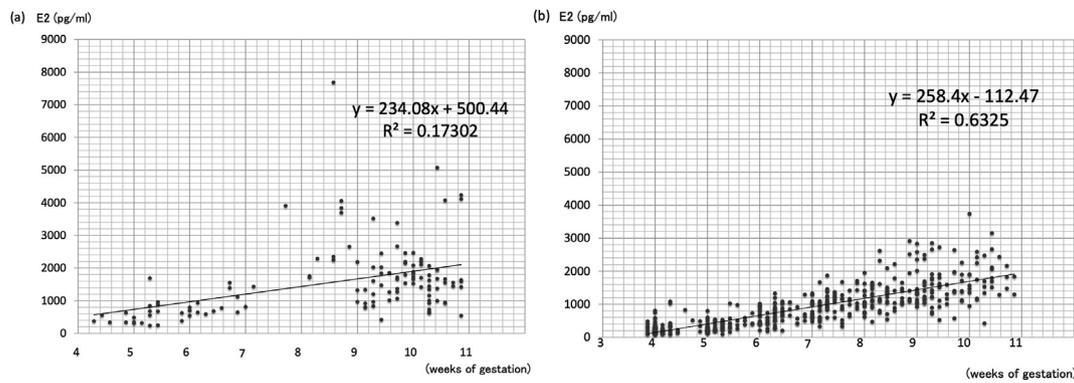


Figure 2. Serum E2 levels during the first trimester in (a) the non-HR group and (b) the HR group.

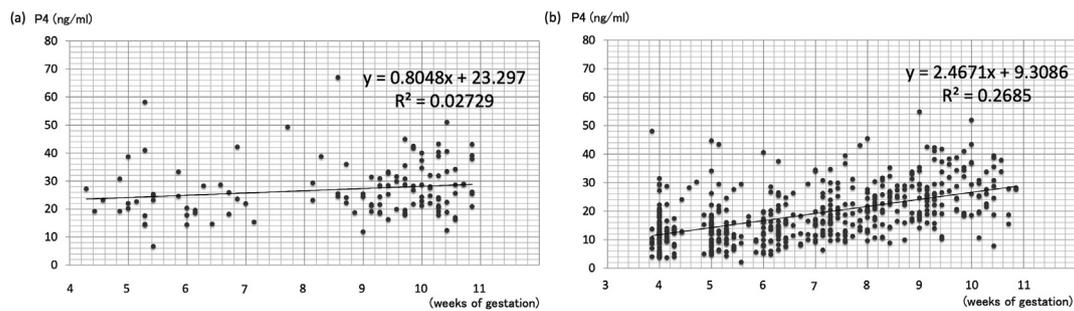


Figure 3. Serum P4 levels during the first trimester in (a) the non-HR group and (b) the HR group.

Table 1. Serum E2 levels over time in the non-HR and HR groups.

	non-HR group (n)	HR group (n)	P value
4 weeks	453.8 ± 137.7 (5)	291.9 ± 200.7 (75)	NS
5 weeks	622.3 ± 379.7 (15)	351.3 ± 173.0 (75)	<0.05
6 weeks	870.1 ± 326.7 (12)	644.1 ± 262.3 (74)	<0.01
7 weeks	2056.3 ± 1635.0 (3)	976.6 ± 342.2 (73)	<0.01
8 weeks	3232.0 ± 1781.3 (10)	1285.7 ± 453.4 (71)	<0.01
9 weeks	1589.3 ± 660.9 (32)	1556.3 ± 587.9 (62)	NS
10 weeks	1790.9 ± 974.6 (42)	1781 ± 649.2 (36)	NS

Data are expressed as mean ± SD, NS; not significant, unit: pg/mL.

The serum P4 level was significantly higher in the non-HR group than in the HR group at 4, 5, and 6 weeks of gestation (4 weeks, $p < 0.01$; 5 weeks, $p < 0.01$; 6 weeks, $p < 0.01$). There was no significant difference in the levels at 7, 8, 9, or 10 weeks of gestation between the groups (Table 2).

Table 2. Serum P4 levels over time in the non-HR and HR groups.

	non-HR group (n)	HR group (n)	P value
4 weeks	23.9 ± 5.1 (5)	14.0 ± 7.8 (75)	<0.01
5 weeks	25.8 ± 12.7 (15)	14.7 ± 8.6 (75)	<0.01
6 weeks	22.7 ± 326.7 (12)	15.4 ± 7.5 (74)	<0.01
7 weeks	28.9 ± 18.0 (3)	18.8 ± 7.6 (73)	NS
8 weeks	30.9 ± 14.1 (10)	22.4 ± 7.4 (71)	NS
9 weeks	25.8 ± 7.2 (32)	26.9 ± 8.9 (62)	NS
10 weeks	28.5 ± 8.8 (42)	27.6 ± 9.3 (36)	NS

Data are expressed as mean ± SD, NS; not significant, unit: ng/mL.

4. Discussion

Although some studies have reported that the use of ART in breast cancer survivors does not significantly affect the prognosis of cancer [7, 8, 9], Venn's report have indicated that if women without breast cancer receive more than six sessions of controlled ovarian stimulation (COS) under ART, the standardized incidence rate of breast cancer increases 1.23-times [10]. Exposure to estrogen causes dose-dependent cellular proliferation and an increase in cancer cell lines estrogen receptor [11]. As serum estrogen levels increase with COS, the risk of recurrence is of particular concern in patients with hormone receptor-positive breast cancer [12, 13]. Recently, COS using gonadotropin-releasing hormone (GnRH) antagonists and aromatase inhibitors was reported to be effective for minimizing any increase in the serum E2 levels [14, 15]. However, when aromatase inhibitors are used for COS, the serum P4 level is maintained at a comparatively high level [16]. Conventionally, progesterone levels do not affect the risk of breast cancer [17], although some studies have reported that progesterone levels may contribute toward breast cancer risk [18, 19]. Therefore, a method in which the serum E2 and P4 levels do not increase above the required levels must be selected when using ART for breast cancer survivors.

In ART, progesterone and a combination of progesterone and estrogen have been reported to be effective for embryo implantation in the endometrium [20, 21]. In a frozen-thawed embryo transfer and during HRT, ovulation does not occur; thus, corpus luteum is not formed and progesterone is not secreted endogenously. If the corpus luteum is removed before 7 weeks of gestation, the serum P4 level suddenly decreases and miscarriage may occur [22]. Thus, luteal support is necessary until at least 7 weeks of gestation. During the natural cycle, luteal support is not necessarily required; however, it is needed if there are luteal phase defects. Many women with decreased ovarian function after neoadjuvant chemotherapy for breast cancer who do not have an ovulatory cycle and whose fertility has declined are likely to require HRT, including the administration of estrogen and progesterone.

The effect of estrogen and progesterone replacement therapies on breast cancer is concerning. Currently, the safety of these replacements is

not known. In this study, we measured the levels of serum E2 and P4 levels in the first trimester of pregnancy in infertile women who did not receive HRT. Our results suggest that in the first trimester, the serum E2 and P4 levels in the HR group were not higher than those in the non-HR group. Thus, the amount of HRT required to establish a pregnancy may not increase the cancer risk associated with hormonal exposure in patients with hormone receptor-positive breast cancer compared to that in those with spontaneous pregnancy.

We found that the serum E2 levels were significantly lower in the HR group than in the non-HR group at 5, 6, and 8 weeks of gestation. Differences at 4 and 7 weeks of gestation may not have been statistically significant owing to the small sample size. In the non-HR group, the levels tended to increase over time (Table 1). At approximately 6–7 weeks of gestation, the main site of hormone production switches from the corpus luteum to the placenta [23], and the levels rapidly increased during this period (Figure 2a). There was no significant difference in the serum E2 levels at 9 and 10 weeks of gestation between the two groups; this may be because estrogen replacement therapy was discontinued in the HR group at 8 weeks of gestation, and in both groups, the main site of hormone production was now the placenta.

In the non-HR group, the serum P4 levels gradually increased between 4 and 10 weeks of gestation to 20–30 ng/mL (Figure 2b), and in the HR group, the level remained at approximately 15 ng/mL, which was significantly lower than that in the non-HR group. Since there was no corpus luteum formation in the HR group, the serum P4 levels would have been solely derived from the transvaginal natural progesterone administered. Although the use of transvaginal natural progesterone results in significantly lower serum P4 levels than those achieved after intramuscular injection of natural luteal hormone, the P4 concentration in the endometrial tissue is significantly higher; hence, the serum P4 level does not reflect the local concentration in the tissue [24, 25]. In practice, the serum P4 levels are reported to be low after the administration of transvaginal natural progesterone, but the P4 concentration in the endometrial tissue is maintained at a higher level [26]. In this study, because few samples were collected from patients in the non-HR group at 7 weeks of gestation, we could not evaluate this aspect. However, as the placenta started to produce hormones by approximately 6–7 weeks of gestation, the hormone levels increased after 8 weeks of gestation in the HR group, and the significant difference between the groups disappeared.

This study has several limitations. First, only a few samples were collected from the patients in the non-HR group. Therefore, we contained patients undergoing medication for ovulation induction in non-HR group. This was unavoidable because the study design involved the use of left-over blood from blood drawn during scheduled hospital visits by women who conceived naturally or after IUI. Second, because this study did not include breast cancer survivors, we were unable to assess the prognosis or risk of recurrence in breast cancer survivors who underwent thawed embryo transfer with HRT of estrogen and progesterone. It is an ethical dilemma not to offer HR to breast cancer survivors when they have a rare opportunity to conceive using ART. Hence, we opted for a study design in which we compared the non-HR and HR groups.

In conclusion, our results suggest that increased serum E2 and P4 levels in the first trimester of pregnancy owing to HRT are lower than the serum E2 and P4 levels noted during the first trimester of spontaneous pregnancy or pregnancy following regular infertility treatment. This suggests that the risk of breast cancer associated with thawed embryo transfer with HRT of estrogen and progesterone may not be greater than that associated with spontaneous pregnancy.

Declarations

Author contribution statement

Ayumu Ito and Yusuke Fukuda: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Mineto Morita: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Yukiko Katagiri: Analyzed and interpreted the data; Wrote the paper.

Funding statement

This study was supported by the Department of Obstetrics and Gynecology, Faculty of Medicine, Toho University.

Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

Acknowledgements

The authors would like to thank to all colleague of the Department of Obstetrics and Gynecology, Reproduction Center, Department of Laboratory Medicine, Toho University Omori Medical Center for the cooperation of the subjects' recruitment and measuring blood samples.

References

- [1] M. Lambertini, N. Kroman, L. Ameye, O. Cordoba, A. Pinto, G. Benedetti, M.B. Jensen, S. Gelber, M. Del Grande, M. Ignatiadis, E. De Azambuja, M. Paesmans, F.A. Peccatori, H.A. Azim, Long-term safety of pregnancy following breast cancer according to estrogen receptor status, *J. Natl. Cancer Inst.* 110 (2018) 426–429.
- [2] M. Lambertini, L. Ameye, A.-S. Hamy, A. Zingarello, Pregnancy after breast cancer in patients with germline BRCA mutations, *J. Clin. Oncol.* 10 (2020) 3012–3023.
- [3] E. Breast Cancer Trialists, C. Group, Articles Comparisons between different polychemotherapy regimens for early breast cancer: meta-analyses of long-term outcome among 100 000 women in 123 randomised trials, *Lancet* 379 (2012) 432–444.
- [4] T. Fréour, P. Barrière, D. Masson, Anti-müllerian hormone levels and evolution in women of reproductive age with breast cancer treated with chemotherapy, *Eur. J. Cancer* 74 (2017) 1–8.
- [5] B.A. Mueller, M.S. Simon, D. Deapen, A. Kamineni, K.E. Malone, J.R. Daling, Childbearing and survival after breast carcinoma in young women, *Cancer* 98 (2003) 1131–1140.
- [6] R.A. Anderson, D.H. Brewster, R. Wood, S. Nowell, C. Fischbacher, T.W. Kelsey, W.H.B. Wallace, The impact of cancer on subsequent chance of pregnancy: a population based analysis, *Hum. Reprod.* 33 (2018) 1281–1290.
- [7] O. Goldrat, N. Kroman, F.A. Peccatori, O. Cordoba, B. Pistilli, O. Lidgaard, I. Demeestere, H.A. Azim, Pregnancy following breast cancer using assisted reproduction and its effect on long-term outcome, *Eur. J. Cancer* 51 (2015) 1490–1496.
- [8] T.N. Sergentanis, A.A. Diamantaras, C. Perlepe, P. Kanavidis, A. Skalkidou, E.T. Petridou, IVF and breast cancer: a systematic review and meta-analysis, *Hum. Reprod. Update* 20 (2014) 106–123.
- [9] E. Rosenberg, A. Fredriksson, Z. Einbeigi, C. Bergh, A. Strandell, No increased risk of relapse of breast cancer for women who give birth after assisted conception, *Hum. Reprod. Open.* 2019 (2019) hoz039-undefined.
- [10] A. Venn, L. Watson, F. Bruinsma, G. Giles, D. Healy, Risk of cancer after use of fertility drugs with in-vitro fertilisation, *Lancet* 354 (1999) 1586–1590.
- [11] A. Cooley, L. Matthews, S. Zelivianski, A. Hardy, J.S. Jeruss, Effect of infertility treatment and pregnancy-related hormones on breast cell proliferation in vitro, *Hum. Reprod.* 27 (2012) 146–152.
- [12] E. Muñoz, N. González, L. Muñoz, J. Aguilar, J.A. García Velasco, Ovarian stimulation in patients with breast cancer, *E Cancermedicallscience* 9 (2015) 504–undefined.
- [13] M. Clemons, P. Goss, Estrogen and the risk of breast cancer, *N. Engl. J. Med.* 344 (2001) 276–285.
- [14] H. Cakmak, A. Katz, M.I. Cedars, M.P. Rosen, Effective method for emergency fertility preservation: random-start controlled ovarian stimulation, *Fertil. Steril.* 100 (2013) 1673–1680.
- [15] K. Oktay, A. Hourvitz, G. Sahin, O. Oktay, B. Safro, A. Cil, H. Bang, Letrozole reduces estrogen and gonadotropin exposure in women with breast cancer undergoing ovarian stimulation before chemotherapy, *J. Clin. Endocrinol. Metab.* 91 (2006) 3885–3890.

- [16] O. Goldrat, C. Gervy, Y. Englert, A. Delbaere, I. Demeestere, Progesterone levels in letrozole associated controlled ovarian stimulation for fertility preservation in breast cancer patients, *Hum. Reprod.* 30 (2015) 2184–2189.
- [17] M. Samson, N. Porter, O. Orekoya, J.R. Hebert, S.A. Adams, C.L. Bennett, S.E. Steck, Progestin and breast cancer risk: a systematic review, *Breast Cancer Res. Treat.* 155 (2016) 3–12.
- [18] H.N. Hilton, N. Santucci, A. Silvestri, S. Kantimm, L.I. Huschtscha, J.D. Graham, C.L. Clarke, Progesterone stimulates progenitor cells in normal human breast and breast cancer cells, *Breast Cancer Res. Treat.* 143 (2014) 423–433.
- [19] C. Brisken, Progesterone signalling in breast cancer: a neglected hormone coming into the limelight, *Nat. Rev. Cancer.* 13 (2013) 385–396.
- [20] M. van der Linden, K. Buckingham, C. Farquhar, J.A.M. Kremer, M. Metwally, Luteal phase support for assisted reproduction cycles, *Cochrane Database Syst. Rev.* 2015 (2015).
- [21] X.M. Zhang, F. Lv, P. Wang, X.M. Huang, K.F. Liu, Y. Pan, N.J. Dong, Y.R. Ji, H. She, R. Hu, Estrogen supplementation to progesterone as luteal phase support in patients undergoing in vitro fertilization, *Med. (United States).* 94 (2015) e459.
- [22] A.I. Csapo, M.O. Pulkkinen, B. Ruttner, J.P. Sauvage, W.G. Wiest, The significance of the human corpus Luteum in pregnancy maintenance, *Am. J. Obstet. Gynecol.* 112 (1972) 1061–1067.
- [23] R. Tal, H.S. Taylor, R.O. Burney, S.B. Mooney, L.C. Giudice, in: K.R. Feingold (Ed.), *Endocrinology of Pregnancy*, Endotext, 2015, pp. 16–25. <https://www.endotext.org>.
- [24] R.J. Paulson, M.G. Collins, V.I. Yankov, Progesterone pharmacokinetics and pharmacodynamics with 3 dosages and 2 regimens of an effervescent micronized progesterone vaginal insert, *J. Clin. Endocrinol. Metab.* 99 (2014) 4241–4249.
- [25] R.A. Miles, R.J. Paulson, R.A. Lobo, M.F. Press, L. Dahmouh, M. V Sauer, Pharmacokinetics and endometrial tissue levels of progesterone after administration by intramuscular and vaginal routes- a comparative study, *Fertil. Steril.* 62 (1994) 485–490.
- [26] Matteo Lambertini, Lieveke Ameye, Anne-Sophie Hamy, Anna Zingarello, High local endometrial effect of vaginal progesterone gel, *J. Clin. Oncol.* 38 (2020) 3012–3023.