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ASSISTED REPRODUCTION TECHNOLOGIES

Hormone concentrations of dominant follicles in the TALES randomized controlled trial comparing letrozole with tamoxifen

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Abstract

Background In this secondary analysis of the TAmoxifen or Letrozole in Estrogen Sensitive tumors (TALES) trial, we aimed to investigate if concurrent administration of letrozole vs. tamoxifen vs. no added treatment affects hormonal composition and size of stimulated ovarian follicles.

Methods TALES is a randomized controlled trial of IVF stimulation for estrogen receptor (ER)–positive breast cancer patients stimulated with gonadotropins and administered concurrent tamoxifen 20 mg or letrozole 5 mg. We analyzed estradiol (E2), testosterone (T), progesterone (P4), follicle stimulating hormone (FSH), luteinizing hormone (LH), and anti-Mullerian hormone (AMH). We used ANOVA/Kruskal–Wallis, logistic, and linear regression models to examine differences in follicular hormone levels, size, and mature oocyte yield between trial arm.

Results We included data from total 246 follicles (94 letrozole, 82 tamoxifen, and 70 control) from 123 unique participants. E2 was lower (letrozole 187.4, tamoxifen 1026.0, control 821.5 ng/mL, $p < 0.01$) and T was higher (letrozole 2489, tamoxifen 571, and control 504 ng/mL, $p < 0.03$) in the letrozole group compared to tamoxifen and control groups, while other hormone levels and follicle size were similar across groups. There were no significant differences in hormone concentrations within the follicle between tamoxifen and control arms. On multivariate logistic regression, there was no significant association of mature oocyte yield by follicle size, hormone levels, or trial arm.

Conclusions Concurrent administration of letrozole with gonadotropins affects follicular E2 and T concentrations compared to tamoxifen/control. Tamoxifen was not associated with any differences in hormone concentrations within the follicle. Mature oocyte yield was similar across groups.

Keywords Letrozole · Tamoxifen · Follicular fluid · Fertility preservation · Estradiol · Testosterone

Introduction

Prior literature has suggested that follicular fluid hormonal milieu may affect oocyte yield and quality [1–3]. The developing ovarian follicle contains a number of compounds including steroid hormones, polypeptide hormones, proteins, reactive oxygen species, antioxidants, and polysaccharides; a complex set of interactions occurs between these numerous substances to nourish a developing oocyte during the follicular phase leading up to ovulation [4–7]. Follicular fluid can also serve as a non-invasive method to assess the environment of the oocyte during in vitro fertilization (IVF) cycles, as this fluid is aspirated at the time of egg retrieval [6, 8]. Literature suggests that the follicular fluid hormonal content may be linked to embryo and pregnancy outcomes in natural conception as well as assisted reproductive technologies (ART) [2, 9–11].

Several studies have reported that higher follicular fluid estradiol (E2) levels are correlated with a variety of IVF outcomes, including fertilization, embryo development, and pregnancy rates [2, 8, 10, 11]. Follicular fluid progesterone (P4) levels have also been linked to IVF

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outcomes such as fertilization, as described by a systematic review of 13 studies [9]. Additionally, other follicular hormones including anti-Mullerian hormone (AMH), follicle stimulating hormone (FSH), luteinizing hormone (LH), and testosterone (T) have also been associated with IVF outcomes [12–15]. However, despite these reports, not all studies have found associations between follicular fluid hormone content and fertilization or pregnancy outcomes, with some reporting null associations [1, 15, 16]. The overall literature on follicular fluid hormonal content and IVF outcomes is limited; existing studies contain considerable limitations including small sample sizes, heterogenous hormone assays, and older data.

Letrozole is an aromatase inhibitor and tamoxifen is an estrogen (E2) receptor modulator that can be concurrently administered during ovarian stimulation in cases where there is theoretical concern about high E2 levels caused by exogeneous gonadotropins. Such cases may include hormone receptor-positive malignancy, transgender patients, history of venous thromboembolism, or symptomatic endometriosis. Though the mechanisms of the two medications are different (letrozole as an aromatase inhibitor and tamoxifen as a selective estrogen receptor modulator), both have been used in ART protocols with similar stimulation outcomes to controls [17–19]. There are limited studies on the effect of these compounds on follicular fluid hormonal content [20–23].

The original TAmoxifen or Letrozole in Estrogen Sensitive tumors (TALES) trial was a randomized controlled trial (RCT) of non-metastatic breast cancer patients that compared ovarian stimulation outcomes for concurrent letrozole versus tamoxifen during gonadotropin stimulation (with a control group of estrogen receptor (ER)–negative patients) [24]. TALES found that ovarian stimulation outcomes were similar across all groups in terms of number of mature oocytes as the primary outcome, as well as total oocytes, total mature follicles, and oocyte maturity rate. In this secondary analysis of the TALES RCT, we aimed to investigate if concurrent administration of letrozole vs tamoxifen (with no added treatment as an ER negative control) affects hormonal composition, size, and maturity of stimulated ovarian dominant follicles. Given studies suggesting a linkage between follicular fluid content of certain hormones and oocyte competence, we focused on the following six follicular fluid hormones in our analysis: E2, T, P4, FSH, LH, and AMH. We hypothesized that follicular fluid E2 would be significantly lower and follicular fluid T would be significantly higher in the letrozole group due to the mechanism of aromatase inhibitor, while other follicular fluid hormones would not necessarily be different between groups.

Methods

This is a secondary analysis of a subset of the TALES RCT of IVF stimulation outcomes for non-metastatic ER-positive breast cancer patients [24]. We compared the following hormones: E2, T, P4, FSH, LH, and AMH for a large subset of patients in the TALES trial who had follicular fluid information available. The hormones studied were chosen based on literature suggesting possible effect on follicular/oocyte development, as described in the "Introduction" section. The primary outcome of the secondary analysis was hormonal concentrations of the ovarian follicles. Secondary outcomes included follicle size and oocyte maturity of these dominant follicles. The TALES trial protocols have been previously discussed and will be summarized in our manuscript. The trial registration, approval, and safety monitoring have been previously described in the original TALES manuscript as well [24]. This trial was approved by the University of California, San Francisco (UCSF) Committee on Human Research.

Study overview and design (original TALES trial)

The original TALES trial was a randomized controlled trial of concurrent administration of tamoxifen versus letrozole with gonadotropins for ER-positive breast cancer patients undergoing controlled ovarian stimulation. Patients were randomized to concurrent administration of tamoxifen 20 mg or letrozole 5 mg (with no added treatment for ER-negative control group), along with the standard gonadotropin dose selected by the overseeing physician depending on patient and cycle characteristics. The TALES trial also included a third control arm of gonadotropin only for ER-negative breast cancer patients [24]. The primary outcome of the TALES trial was number of mature oocytes during one ovarian stimulation cycle. The primary outcome of the original TALES trial was the number of mature metaphase II (MII) oocytes in the trial arms.

Study population and inclusion/exclusion criteria

The study population included women 18–44 years of age with a new diagnosis of non-metastatic breast cancer who planned to undergo ovarian stimulation for the purposes of fertility preservation. Patients were excluded if they had previously undergone chemotherapy, had recurrent breast cancer, stage IV breast cancer at diagnosis, oncologist concerns about participation, or any other significant disease, illness, or psychiatric disorder that would have interfered with patient safety or participation in the study.

Patients with cancer were enrolled at their initial fertility preservation consult at a single academic center from June 2016 to September 2020.

Ovarian stimulation and follicular fluid analysis protocol

All cycles utilized a random-start GnRH antagonist protocol, with the initial dosage of gonadotropins (Follistim, Merick; Gonal-F, EMD-Serono; and/or Menopur, Ferring) selected by the overseeing physician based on the patient's age, body mass index (BMI), and ovarian reserve as determined by antral follicle count or and/or AMH hormone level. GnRH antagonist (0.25 mg ganirelix acetate, Organon; or 0.25 mg cetrotide, EMD-Seron) was administered when lead follicle reached > = 12 mm to prevent premature ovulation of the cohort. Gonadotropin dose was titrated as each monitoring appointment by the overseeing physician based on growth of follicles; for the tamoxifen and control groups, serum E2 level was also used to titrate gonadotropin dose. Medications were titrated with the goal to maximize mature oocyte yield while minimizing risk of ovarian hyperstimulation syndrome.

For patients randomized to letrozole or tamoxifen, the medication was administered starting the first day of gonadotropin stimulation and the last dose was taken on the day of trigger. The tamoxifen 20 mg dose was not changed during the course of stimulation in accordance with prior literature [17], while letrozole was titrated up to 10 mg/day in some patients to keep E2 levels below a typical physiologic peak of 500 pg/mL which may have theoretical benefit. Serum E2 levels were assayed at each monitoring appointment and used to help titrate gonadotropin dose for tamoxifen and control arms as described above.

When the lead follicle reached approximately 18 mm (for the tamoxifen/control groups) or 20 mm (for the letrozole group) and when the general cohort was > 13 mm, oocyte maturation was induced with sliding scale hCG (1500 to 10,000 IU subcutaneously) and/or 4 mg leuprolide acetate subcutaneously. Transvaginal oocyte retrieval was performed 36 h after administration of trigger. At the time of transvaginal oocyte retrieval, the visually appearing largest follicle on each side was aspirated in two separate collection tubes, and the collection tube was then switched to aspirate the remainder of follicles on both sides. Switching the collection tube after aspiration of the largest follicle helped ensure no contamination from other follicles. The volume of aspirated largest follicle fluid was then recorded, and the sample was stored. The rationale for comparing the 2 dominant follicles was based on the fact that we wanted to aspirate the visually appearing largest follicle on each side and flush each follicle in its own tubes. After retrieval, cumulus cells were stripped from oocytes at 2–3 h and cryopreservation was performed of either mature oocytes or embryos (D3/D5), depending on patient preference and overall cohort quality.

Before running samples, follicular fluid assays were calibrated to known standards and validated by serial dilution. The following hormone concentrations were quantified in batch and duplicate and measured with commercially available automated chemiluminescent immunoassays on the Roche cobas e411: E2, P4, T, FSH, LH, and AMH. As previously described, these hormones were selected, guided by published literature, because of either their possible or established association with the outcome of interest or to each other. Each test was run with three controls of low, medium, and high concentrations. Dilutions were performed before measurement of E2 (1:1,000) and P4 (1:1,000), depending on the calibration range. The intraassay coefficient of variations were E2 (24)%, P4 (26)%, and T(8)%. High or low results were repeated with appropriate dilution. The published serum data for Roche intraassay coefficient of variations for our range of concentrations are E2 (4.6)%, P4 (2.1)%, T(1.5)%, LH(0.8)%, FSH (1.8)%, and AMH(1.4)%.

Statistical analysis

We used t-tests, ANOVA, and Kruskal–Wallis tests to compare follicular fluid concentrations of the six hormones of interest for the bilateral dominant follicles in each cycle. Because the data regarding follicular fluid was not normally distributed, a non-parametric Kruskal–Wallis approach was used to display the primary outcome. Comparisons were first made for the randomized arms of letrozole versus tamoxifen, and then across the three arms including the control arm. Based on results of the initial analysis and the mechanism of the estrogen modulator studied, a secondary analysis was done to compare the tamoxifen and control arms. Follicular fluid hormone concentrations were the primary outcome of our analysis.

We also used t-test and ANOVA to examine differences in follicle size (calculated from follicular volume assuming a spherical shape) and Fisher's exact test to compare mature oocyte yield among dominant follicles across groups. We then used logistic regression to examine the relationship between these follicular fluid concentrations and mature oocyte yield and linear regression to study relationship between hormone levels and follicle size between trial arms. Multivariate analyses were performed to include all parameters of interest for dominant follicles (trial arm, follicles size, FSH, LH, E2, P4, T, AMH). Demographic and ovarian reserve factors were not included in the multivariate analysis due to the randomized controlled trial format. We analyzed each follicle individually (even those from the same patient) given difference in sizes, in order to investigate hormonal concentration, in accordance with prior literature on follicular fluid correlations [25]. As this was a secondary exploratory analysis with investigation

of multiple outcomes (with uncertain effect size expectations given its exploratory nature), a post-hoc power analysis was not calculated; however, we included all of the follicles we had access to from the TALES trial. The original TALES trial found that the type of trigger and oocyte maturity was similar in both groups, so the effect of the trigger was not further investigated in our analysis as we were performing a secondary analysis of the original RCT. All tests were 2-sided with significance at the alpha = 0.05 level. Data analysis was performed in STATA version 16 (Stata Corp, College Station, TX).

Results

This analysis included data from total 246 follicles from 123 unique participants (47 letrozole, 41 tamoxifen, 35 control). This study was a subset of the original TALES trial of 137 patients (which included 51 letrozole, 45 tamoxifen, 38 control), as 14 patients in the TALES trial did not have follicular fluid available for analysis. Baseline characteristics are displayed in Table 1. As seen in the original TALES trial, age was significantly different between the groups, with the tamoxifen group having a higher average age of 35.8 (SD 4.9) years, compared to 33.9 (SD 4.4) for the letrozole group and 32.2 (SD 3.4) for the control group. The groups were similarly distributed in terms of BMI, baseline antral follicle count, and the percentage undergoing oocyte cryopreservation (68% for letrozole, 69% for tamoxifen, and 76% for control, with the remainder undergoing embryo cryopreservation).

In terms of cycle characteristics, the three trial arms were also similar in terms of total gonadotropin dose, number of stimulation days, and number of mature oocytes for the cohort. The peak serum E2 level during stimulation was significantly different across groups with the lowest value observed in the letrozole group (letrozole 643.4, tamoxifen 3164.8, control 2621.2 pg/mL, $p < 0.001$). Peak serum E2 was also significantly higher in the tamoxifen group compared to control ($p = 0.04$).

Table 2 displays the values of follicular fluid concentrations of the six hormones of interest across the three trial arms. E2 was lower (letrozole 187.4, tamoxifen 1026.0, control

Table 1 Baseline characteristics by trial arm

	Letrozole	Tamoxifen	p-value (letrozole vs tamoxifen)	Control	p-value (all groups)
Number	94	82		70	
Age	33.9 (4.4)	35.8 (4.9)	**0.006**	32.2 (3.4)	**< 0.001**
BMI	23.7 (4.3)	24.3 (5.5)	0.39	23.8 (6.4)	0.72
AFC	15.0 (8.3)	15.8 (13.1)	0.63	13.8 (5.9)	0.45
Percentage egg cryo (vs embryo cryo)	64% (68%)	55% (69%)	0.93	52% (76%)	0.46
Total gonadotropin dose (IU)	2298 (693)	2394 (1098)	0.49	2475 (1024)	0.49
Number of stimulation days	10.3 (1.3)	10.0 (1.5)	0.26	10.0 (1.7)	0.46
Peak serum E2 (pg/mL)	643.4 (304.2)	3164.8 (1772.9)	**< 0.001**	2621.2 (1448.5)	**< 0.001**
Number MIIs in cohort	11.5 (7.5)	12.2 (8.9)	0.58	12.5 (6.7)	0.71

Legend: Mean (standard deviation) for all values except for percentage egg cryopreservation. Significant *p*-values denoted in bold

BMI body mass index, *AFC* antral follicle count, *IU* international units

Table 2 Follicular fluid hormonal concentrations, size, and oocyte maturity by trial arm

Follicular fluid hormone	Letrozole	Tamoxifen	p-value (letrozole vs tamoxifen)	Control	p-value (all groups)
FSH (mIU/L)	100 (46, 161)	135 (70, 176)	0.54	120 (58, 186)	0.12
LH (mIU/L)	118 (69, 159)	122 (69, 166)	0.79	89 (42, 145)	0.13
AMH (ng/mL)	71 (43, 115)	76 (40, 124)	0.60	74.5 (47, 138)	0.71
P4 (ng/mL)	27,455 (17,575, 42.720)	34,935 (22,830, 41,795)	0.22	33,353 (20,150, 42,188)	0.47
E2 (ng/mL)	187.4 (95.7, 369.4)	1026.0 (459.1, 1496.0)	**< 0.001**	821.5 (424.6, 1363.8)	**< 0.001**
T (ng/mL)	2489 (142, 4845)	571 (346, 900)	**0.017**	504 (333, 846)	**0.03**
Follicle size	20.0 (2.6)	19.1 (3.2)	0.93	20.0 (3.4)	0.11
MIIs retrieved	52 (55%)	39 (56%)	0.18	37 (45%)	0.33

Legend: Values are displayed as median (IQR) for all hormones, except mean (SD) for follicle size and number for mature egg. Dominant follicles only were included in the analysis. Significant *p*-values denoted in bold

821.5 ng/mL, $p < 0.01$) and T was higher (letrozole 2489, tamoxifen 571, and control 504 ng/mL, $p < 0.03$) in the letrozole group compared to tamoxifen and control groups, while other hormone levels and follicle size were similar across groups. There were no significant differences between tamoxifen and control arms. The values of follicular fluid FSH, LH, AMH, and P4 concentrations were statistically similar, both between randomized groups and across all three trial arms. On a secondary comparison between tamoxifen and control arms, we found that all follicular fluid hormones (including E2 and T) were similar between the two groups.

The mature oocyte yield was similar between randomized groups and overall (including control group). We also found (Table 3) there was no significant association of odds of mature oocyte for a given follicle size, hormone levels, or trial arm ($p > 0.05$ for all parameters studied). In addition, there was no significant association of lead follicle size at the time of retrieval with hormone levels or trial arm ($p > 0.05$ for all parameters) (Table 4).

Table 3 Logistic multivariate regression for odds of mature oocyte

Parameter	OR	95% CI	P-value
Arm			
Letrozole	Reference		
Control	1.79	0.68 to 4.70	0.24
Tamoxifen	1.72	0.62 to 4.71	0.30
Follicle size	1.04	0.96 to 1.14	0.34
FSH	1.00	1.00 to 1.00	0.97
LH	1.00	1.00 to 1.01	0.89
E2	1.00	1.00 to 1.00	0.09
P4	1.00	1.00 to 1.00	0.42
Testosterone	1.00	0.99 to 1.00	0.49
AMH	1.00	0.99 to 1.01	0.76

Legend: Multivariate analysis adjusted for all parameters displayed. Dominant follicles only were included in the analysis

Table 4 Linear multivariate regression for follicle size

Parameter	Coefficient	95% CI	p-value
Arm			
Letrozole	Reference		
Control	−1.03	−2.41 to 0.35	0.14
Tamoxifen	−0.33	−1.79 to 1.12	0.65
FSH	−0.003	−0.01 to 0.003	0.37
LH	−0.004	−0.01 to 0.004	0.30
E2	0.00	−0.00 to 0.00	0.62
P4	0.00	−0.00 to 0.00	0.81
Testosterone	0.00	−0.00 to 0.00	0.92
AMH	−0.003	−0.01 to 0.01	0.52

Legend: Multivariate analysis adjusted for all parameters displayed. Dominant follicles only were included in the analysis

Discussion

In summary, for a cohort of non-metastatic breast cancer patients undergoing gonadotropin stimulation with concurrent letrozole, tamoxifen, or no added medication, we found that the follicular fluid hormonal milieu was similar for the hormones studied (FSH, LH, AMH, P4), with the exception of E2 (significantly lower in the letrozole group for both follicular fluid and serum hormonal levels) and T (significantly higher in the letrozole group). These relationships were found both between randomized groups, as well as across all three trial arms. Additionally, we confirmed in this subgroup of the original TALES trial that despite this difference in follicular fluid E2, there were no differences across groups in terms of stimulation outcomes like gonadotropin use, duration of ovarian stimulation, and mature oocyte yield. Tamoxifen and control groups had similar follicular hormonal profiles and stimulation results across all parameters studied, which has not been previously reported in literature.

Studies have found that the hormonal content of follicular fluid may be linked to pregnancy outcomes, both in natural conception and with ART. A systematic review of 13 studies found that follicular fluid P4 levels were significantly higher in normal fertilization than in failed fertilization for both conventional IVF and ICSI cycles, though varied P4 measurement methods were used by the included studies [9]. A study of 64 follicles from women who failed to conceive after IVF and 33 follicles of women who conceived after IVF found that higher follicular fluid E2 levels correlated with successful fertilization and enhanced cleavage rate of oocytes [8]. Another older study of 19 prevoulatory oocytes found that follicular fluid E2 and P4 were both markers of oocyte quality based on increased fertilization, cleavage, and pregnancy rates within a certain band of E2 and P4 follicular fluid levels [2]. Other studies have also suggested that follicular fluid E2 levels may be correlated with IVF outcomes, including oocyte yield, pregnancy outcomes, and oxidative stress [10, 11]. Our study only found changes in follicular fluid levels of E2 and T (with the use of aromatase inhibitor compared to estrogen receptor modulator), without a difference in other follicular fluid values.

Follicular fluid also contains AMH, which is a glycoprotein secreted by the granulosa cells and is a commonly used serum marker of ovarian reserve [26–28]. AMH has been studied as a follicular fluid marker, as animal and human studies have both shown that atretic follicles fail to secrete AMH [29–31]. Several small studies have found that fertilization, embryo development, and pregnancy outcomes may be linked to follicular AMH levels [12–14]. Studies have also found differences in follicular fluid hormonal content for PCOS patients, with one study of 42 patients reporting significantly increased AMH and decreased FSH levels in

follicular fluid (from both small and large follicles) of PCOS patients; this study also reported that follicular AMH levels were significantly lower in patients who began a pregnancy [15]. Our study found no differences between follicular fluid concentrations of AMH between the three study groups, suggesting that the use of estrogen modulators does not affect this hormone. These results are helpful for counseling given possible linkage of follicular AMH to study outcomes, though more research is needed to validate these results and the linkage to longer-term outcomes.

It has been suggested the hormonal milieu of follicular fluid differs in natural cycles compared to gonadotropin stimulated IVF, suggesting that exogeneous gonadotropin stimulation affects the relative hormonal content of follicular fluid [16]. However, not all studies have found relationship between follicular fluid hormonal content and oocyte competence. A study of 206 follicles of 35 women undergoing controlled ovarian stimulation reported that follicular fluid steroid hormone content was correlated with follicular size, but not oocyte maturation/ability to fertilize [1]. The previously referenced study of 42 patients found no significant differences in follicular E2, androstenedione, hCG, and P4 levels between PCOS and non-PCOS patients (despite reporting differences in follicular AMH and FSH); there were also no differences in these levels between patients who did and did not achieve a pregnancy [15]. Our study did not find differences in follicular fluid AMH, FSH, LH, or P4 in gonadotropin-stimulated cycles, which suggests that E2 modulators do not disrupt these other follicular fluid hormones in IVF cycles.

With regards to estrogen modulators, letrozole inhibits the conversion of androgen to E2 as an aromatase inhibitor, which causes decreases in serum E2 levels. Studies on letrozole when used with gonadotropins for fertility preservation have shown no long-term increased cancer risk [17, 19, 32–35]. Tamoxifen has a different mechanism as a selective estrogen receptor modulator and does not strongly impact serum E2 levels due to its action at the receptor level. These compounds may be expected to have similar activity on follicular fluid as compared to serum levels, though follicular fluid impact by these medications has limited studies. A prospective study of 23 breast cancer patients treatment with letrozole during ovarian stimulation (compared with 24 infertile patients) found that the letrozole group had significantly lower follicular E2 and higher T levels (similar to our study), though embryo outcomes were not reported [20]. A pilot study of 147 low-responder patients found that treatment with letrozole 2.5 mg with a high-dose FSH/HMG-antagonist regimen had significantly higher levels of follicular fluid T and androstenedione in the letrozole group, and that letrozole-treated patients had similar oocytes retrieved with higher implantation rates [21]. An RCT showed that letrozole with gonadotropins (compared to gonadotropins alone) had no effect on premature P4 rise but increased serum P4 levels in the mid-luteal phase, though follicular fluid levels were not studied [36]. The literature on tamoxifen is even more limited as this is a less commonly used medication in infertility protocols. An older study of 34 patients undergoing laparoscopic follicle aspiration compared 19 women given 80 mg tamoxifen 4 h prior to trigger with 15 controls (though not given concurrently during stimulation) [22]. This study found similar fertilization rates between the tamoxifen and control group, as well as similar follicular fluid E2, P4, and androstenedione concentrations. Studies have also examined the use of aromatase inhibitors in sequential or priming protocols, rather than concurrent administration, with a small randomized controlled trial of sequential letrozole with hMG in 53 patients, finding that the letrozole group had higher follicular fluid concentrations of T, androstenedione, FSH, and AMH with lower miscarriage rates [23]. Prior studies using aromatase inhibitors for androgen priming in the setting of diminished ovarian reserve found increased follicular T but no improvement in pregnancy outcomes [37, 38]. The overall literature on letrozole/tamoxifen effects on follicular fluid is extremely limited with small sample sizes.

Our study showed that concurrent administration of tamoxifen results in similar follicular fluid hormone concentrations with control, while letrozole affects both follicular fluid E2 and T without creating differences in other follicular fluid hormone changes. This result was not unexpected given the mechanism of letrozole, though our study is the first to confirm this finding in a randomized trial format for concurrent administration of gonadotropins with estrogen modulators. Despite these differences in follicular fluid E2 and T levels, no change in mature oocyte yield was found between the groups. We also found that on multivariate regression analysis, follicle size and maturity were not related to follicular fluid hormonal levels for the largest follicles in the cohort. Our findings suggest that decreased E2 level and increased T level in the follicular fluid via an aromatase inhibitor mechanism do not affect oocyte maturity; however, it is possible (as suggested by existing literature) that these follicular fluid hormone levels may be linked to oocyte competence in natural or IVF cycles where estrogen modulators are not used.

Strengths and limitations

Strengths of our study include relatively large sample size, novelty of the subject matter, detailed information on six different follicular fluid hormone levels, secondary analysis of a randomized controlled trial, and completion of the study at a single academic center which allowed for use of consistent hormone assays. However, our study had a number of limitations. Our analysis was limited to breast cancer patients only, which may limit generalizability to other populations such as

non-cancer patients who may have reason to use E2 modulators during ovarian stimulation (including for endometriosis, transgender patients, or those with venous thromboembolism). This study was also a secondary analysis; as such, the outcomes studied were not part of the original outcomes of the TALES trial, and the original TALES trial was powered to detect a set of different outcomes. Additionally, while the sample size is relatively large for a fertility preservation study focused on cancer patients, this is still relatively small for studies of the overall fertility preservation population. We also studied only the largest follicle on either side of the cohort, and it is possible that different types of relationships would be found among follicles that are smaller, particularly below the threshold of maturity. Additional limitations of the original TALES trial have been previously described in literature, some of which are relevant to this study (including inability to blind physicians and patients to treatment arm, lack of information on cancer genes which may affect ovarian reserve, and the fact that we were not able to study the important outcomes of disease recurrence/progressive and live birth) [24]. Our study does not currently have follow-up to allow examination of embryo or pregnancy outcomes. However, the TALES trial is still ongoing, and this is an area of future investigation.

Conclusion

In conclusion, concurrent administration of letrozole with gonadotropins decreases follicular E2 and increases T concentrations compared to tamoxifen/control. In our study, tamoxifen follicular fluid hormone concentrations are statistically similar to control. Mature oocyte yield is similar across groups regardless of these differences in follicular fluid. These results are useful for counseling ER-positive breast cancer patients, as well as other patients who may use either type of estrogen modulators during ovarian stimulation, though more study is needed into longer-term pregnancy and cancer outcomes. Areas for future study include investigation of this question in a larger sample size/non-fertility preservation patients, variation of follicular fluid hormonal profile with a larger range of follicular sizes, and the study of additional hormones, cytokines, and immune modulators in follicular fluid with and without concurrent administration of estrogen modulators.

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Author contribution MR, JL, and AW were involved in study conception and design. AW performed the data analysis. MR, AW, FJH, and MKA performed data interpretation. AW wrote the initial draft of the manuscript. All authors contributed to additional data interpretation and final approval of the manuscript.

Declarations

IRB: This study has obtained IRB approval through University of California, San Francisco.

Conflict of interest The authors declare no competing interests.

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