Investigating Menopausal Hormone Effects on Fat Metabolism

-A Preliminary Investigation

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Author Note

"Maybe that's enlightenment enough: to know that there is no final resting place of the mind; no moment of smug clarity. Perhaps wisdom...is realizing how small I am, and unwise, and how far I have yet to go."

-Anthony Bourdain

The six weeks of my research placement at University of Leeds formed a unique and invaluable experience for my growth as both an aspiring researcher and an explorer. I now deeply resonate with Anthony Bourdain's aforementioned quote but not only in the context of travelling as he intended, but also research. Completing my research project gave me some sense of accomplishment, but mostly made me realize 'how far I have yet to go'. I am incredibly grateful to The Laidlaw Foundation for presenting this research opportunity, as well as funding me generously to travel to the United Kingdom. Additionally, I extend my gratitude to my supervisor, Dr. Amanda MacCannell, who guided me throughout the process and made every day an important learning opportunity.

Abstract

Menopausal transition is associated with a shift towards central adiposity and increased percentage of harmful visceral fat, which subsequently increase risks of cardiovascular and metabolic diseases. The effects of hormonal decline during menopausal transition on lipid metabolism at a cellular level are not well known. This study investigated the changes in lipid accumulation in mature human adipocytes under different hormone concentrations to better understand their roles in causing menopausal adiposity. Adipocytes were treated with physiologically relevant concentrations of estrogen and progesterone individually as well as both combined, mimicking concentrations of the hormones at the mid-luteal phase of the menstrual cycle and post-menopausal state. Lipid uptake was measured through Oil Red O staining and absorbance quantification. Premenopausal hormone concentrations (of estrogen and progesterone together) significantly suppressed lipid accumulation, while the postmenopausal concentrations resulted in lipid quantity indistinguishable from the control. Upon observing the hormones individually, high concentrations of estrogen resulted in the greatest lipid suppression, while the role of progesterone was dose-independent and ambiguous. Additionally, the HRT-mimicking condition recreated the same effect on lipid profile as the premenopausal hormone levels, demonstrating its efficacy on preventing postmenopausal adiposity.

Keywords: menopause, estrogen, progesterone, obesity, HRT, cardiometabolic disease

1. Introduction

Menopause, defined as the point after which ovarian function stops, results in the serum concentrations of the steroid sex hormones estrogen and progesterone to decrease drastically, which is accompanied by many adverse vasomotor symptoms (such as hot or cold flushes, night sweats, palpitation, etc. caused by changes in blood vessel dilations), psychological shifts that affect daily lives, and physiological changes (Davis et al., 2023). This includes increased fat accumulation, which can lead to obesity and subsequent cardiometabolic diseases – for instance, cardiovascular disease risk notably increases later in women than men, around the period of menopausal onset (Anagnostis, 2022; Fasero & Coronado, 2025). Postmenopausal women have a shift in adiposity with more central adiposity, which means that fat tends to be stored in abdominal regions, whereas fat stored in the lower body decreases relative to premenopausal women (Genazzani, 2006). The increase in percentage of visceral fat specifically is harmful since it is known to be directly correlated to incidence of obesity-related cardiometabolic diseases, as it actively releases inflammatory molecules and resists lipolysis (Elffers et al., 2017; Ibrahim, 2009; Potter & Friedl, 2025).

Since the drastic postmenopausal decline of hormones can be offset to some extent by Hormone Replacement Therapy (HRT), it logically 'attenuates' harmful fat accumulation; studies have shown that women taking HRT had similar fat distribution to premenopausal women than postmenopausal women of similar age (Genazzani, 2006; Tchernof, 1998). In a study comparing HRT treated women and untreated women, visceral fat was reduced in the HRT-treated group (Sumino et al., 2003). Given that higher visceral fat percentage leads to higher risk of cardiometabolic disease, HRT can serve as a preventive measure that is most effective in the first ten years of menopause, given that it has been assessed that the benefits overweigh the risks for the patient (Giannini, 2018).

There is evidence that estrogen has catabolic effects on lipid metabolism – administering estrogen to mice results in lower weight gain relative to those without, despite having a high fat diet and increase in fatty acid oxidation due to estrogen has been observed in the liver (Yoon, 2009; Zhu et al., 2012). Conversely, there is limited understanding on the effect of progesterone on lipid metabolism due to mixed results. Progesterone has

been observed to promote lipid synthesis in adipocytes but also to have no significant effect on lipid profile in other experiments - some researchers have suggested that this may be because adipocytes metabolize progesterone into inactive metabolites, which leads to reduced amounts of active hormone available to act on the lipid profile in the first place (Lacasa et al., 2001; Mešalić et al., 2008; Zhang et al., 2009).

This study investigated the effects of physiologically relevant menopausal hormone levels on mature human adipocytes to improve the understanding of their direct effects on lipid accumulation within an adipocyte. Additionally, the intrinsic cellular effect of HRT was also investigated to probe further into its efficacy as a preventive measure for obesity during menopausal transition. An in-vitro experimental study is an important development in this topic as most knowledge on postmenopausal obesity is based on population data from observational studies. Moreover, it is integral to observe realistic effects on human adipocytes as results seen in experiments with mice may not apply to human mechanisms.

2. Methodology

The study was initiated by preparing the adipocytes to test on - commercially available female human primary white preadipocytes were acquired and cultured in growth media inside T75-flasks until confluent. Confluency is an approximate measure of the surface area of the vessel covered by the proliferating cells. Cell proliferation was also supported by splitting the preadipocytes during the growth period into experimental 24-well plates, as cells may stop dividing due to contact inhibition when they get too crowded. After sufficient confluency of 85% was observed, differentiation was induced to create mature adipocytes, as shown in Figure 1. Ideally differentiation should be induced on 100% confluent cells, but practically cells do not always reach that level.

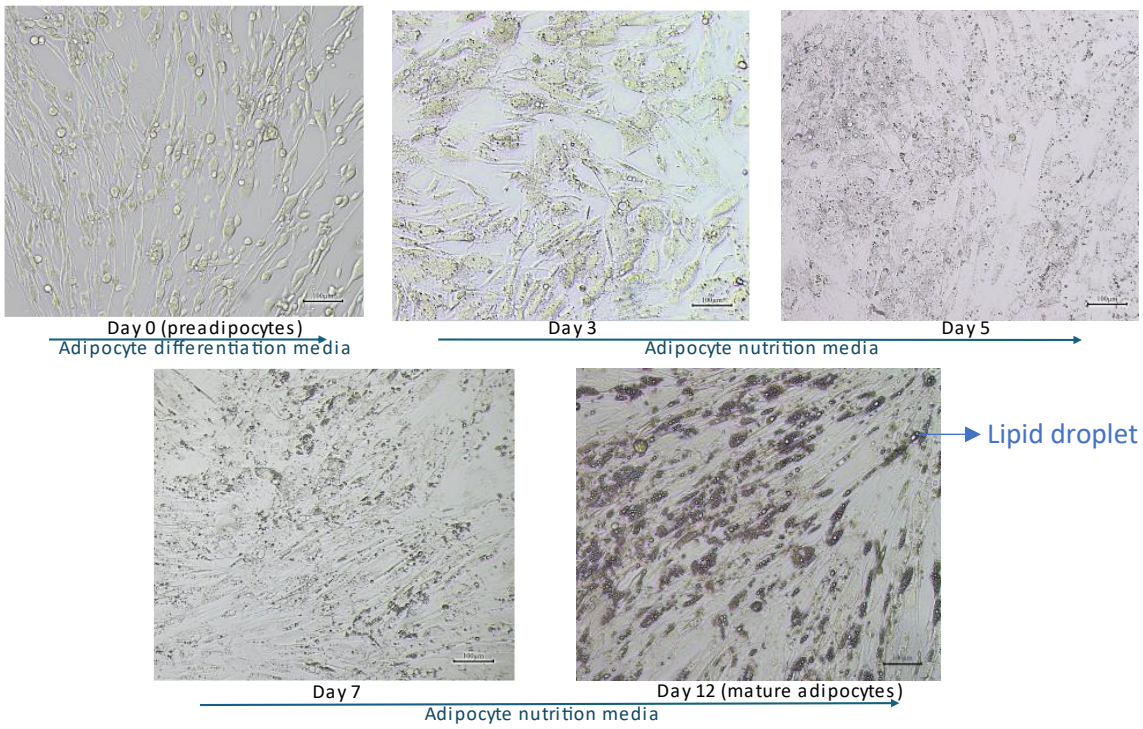

Fig 1: Result of adipocyte differentiation over 12 days
The figure shows differentiating adipocytes at 20x magnification under a light microscope. Differentiation media is added to preadipocytes on Day 0 to start the process. Differentiation into mature adipocytes is visible by the rounding of spindle-like cells and the development of abundant lipid droplets.

2.1) Hormone treatments: Adipocytes were treated with different hormone concentrations starting on day 3, after differentiation began, ensued by careful observation of differences in lipid quantity (which would indicate hormonal impact on lipid metabolism). The setup used to execute this is shown in Figure 2.

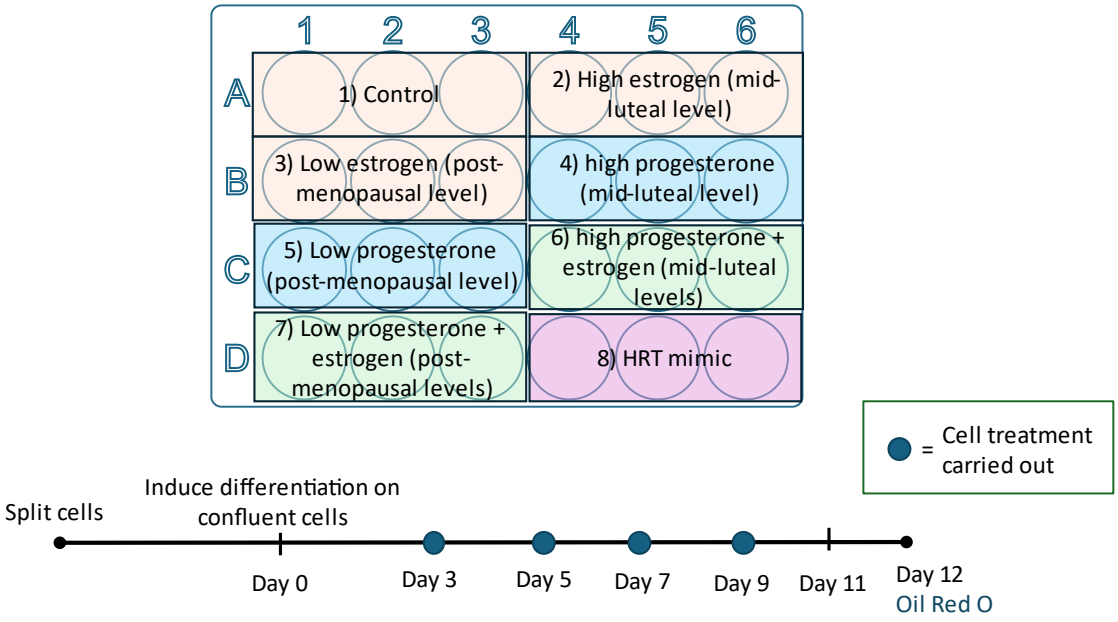

Fig 2: 24-well plate design and summarized research timeline
The figure visualizes the plate design used for the mature adipocyte samples. Adipocytes in three wells were grouped together to be treated with each hormone concentration. They were treated on Day 3, Day 5, Day 7 and Day 9.

The adipocytes in the control wells were treated with only nutrition media (containing no hormones), so they act as a reference of 'normal' lipid accumulation. The estrogen treatments were produced using 17β-Estradiol (also known as E2), which is the major, abundant estrogen in the body (Nilsson et al., 2001). The progesterone treatments were made up of norethisterone acetate (also known as NETA), which is a synthetic hormone that imitates the actions of natural progesterone (Huvinen et al., 2020). Treatments with individual estrogen and progesterone in conditions 2-5 allowed investigation of their separate roles. Condition 7 represents a post-menopausal environment using the low, plateaued combined hormonal concentrations, while condition 6 represents the contrasting pre-menopausal environment.

For the 'high' levels of estrogen and progesterone, serum concentrations specifically during the mid-luteal phase of the menstrual cycle were used. Fat metabolism seems to be promoted during the luteal phase, and the mid-luteal phase marks a point in which both estrogen and progesterone levels are elevated simultaneously (D'Eon et al., 2002). Thus, it was reasonable to select mid-luteal serum concentrations to highlight the premenopausal effect

on lipid accumulation most clearly, and to mimic a realistic physiological condition in condition 6 where the hormones are co-secreted and work together.

After determining the required concentrations of each hormonal treatment, the fat-soluble steroid hormones were dissolved in ethanol (which is an appropriate organic solvent) and diluted serially in PBS buffer. The final concentration was reached after mixing the solutions together with nutrition media.

Condition	Contents of the treatment	Concentration of hormones
1) Control	Nutrition media	----------
2) High estrogen	17β-Estradiol Nutrition media	137.5 pg/mL[a]
3) Low estrogen	17β-Estradiol Nutrition media	11pg/ml[b]
4) High progesterone	Norethisterone acetate Nutrition media	12.33 ng/mL[a]
5) Low progesterone	Norethisterone acetate Nutrition media	0.2ng/ml[c]
6) High estrogen and high progesterone	17β-Estradiol Norethisterone acetate Nutrition media	137.5 pg/mL 12.33 ng/mL
7) Low estrogen and low progesterone	17β-Estradiol Norethisterone acetate Nutrition media	11pg/ml 0.2ng/ml
8) HRT mimic	17β-Estradiol Norethisterone acetate Nutrition media	44 pg/ml[d] 0.47ng/ml[d]

Table 1: Summary of the cell treatment solutions

Note. *[a]Median mid-luteal serum concentrations of estrogen and progesterone from Anckaert et al. (2021). [b]Post-menopausal concentration of estradiol from Stanczyk et al. (2022), [c]progesterone from Henderson et al. (2013). [d]Concentrations following orally administered combined HRT (TX-001HR) from Lobo et al. (2019).*

2.2) Oil Red O protocol: Lastly, an Oil Red O staining protocol was executed on Day 12. To carry out the staining, culture medium was aspirated from the wells of the plate shown in Fig 2, washed with PBS buffer, then the cells were fixed using 4% formaldehyde. 50mL of working solution was made using three-fifths Oil Red O stock solution mixed with distilled water, which was filtered right before usage to remove crystals. The fixed cells were completely covered in this solution and left on a rocker for 45 minutes to allow them to take up the Oil Red O.

After washing the cells with distilled water, the excess Oil Red O dye was removed, only leaving stained lipid droplets inside the adipocytes which were clearly visible when imaged under a microscope. This allowed us to visually compare the different groups of adipocytes. Next, the bound Oil Red O was extracted through dye elution (washing out the Oil Red O inside the cells through dissolution with 100% isopropanol), pipetted into a 96-well plate and placed inside a microplate reader set at 500 nm. The amount of dye bound to lipid inside the cells indicates the amount of lipid accumulation, which was quantified by measuring the relative absorbances of extracted dye.

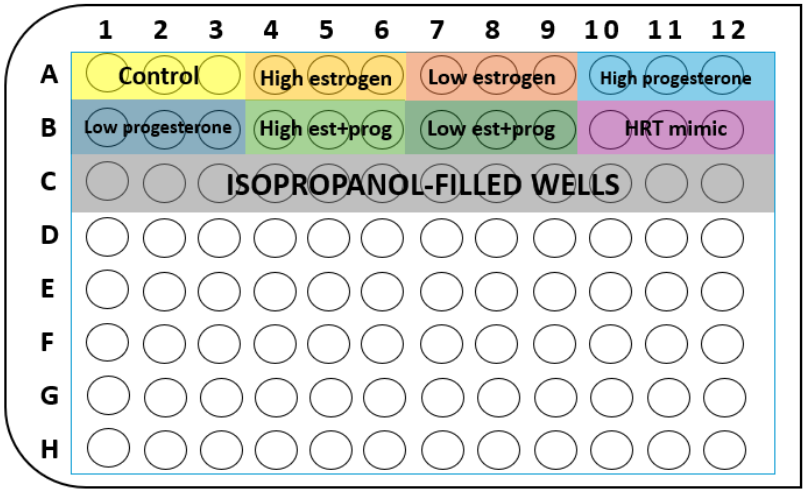

Fig 3: Visual of the 96-well microplate used for the eluted Oil Red O quantification
Three wells were filled with the eluted dye from each condition, corresponding to the three wells in the 24-well plate containing the adipocytes. The wells containing isopropanol only provided blank readings. Blank readings are subtracted from the data before calculating the mean absorbance.

2.3) Statistical analysis: The purpose of statistical analysis was to determine whether the difference in mean absorbances was significant. Assuming normal distribution, ordinary one-way ANOVA test was chosen as there were 8 independent groups of continuous data with homogeneity in variance. The normality had to be assumed since there was only n=3 (per group). To further justify the ANOVA test, Brown-Forsythe test was used to confirm that the difference in variability across the groups is not significant. The required homogeneity in variance would be proven by an F-value close to 1 and $p>0.05$.

Additionally, a post-hoc Tukey's multiple comparisons test was carried out to compare the groups in pairs (in order to point out which specific pairs are significantly distinct). This test is imperative for the main objective of comparing the pair of pre-menopausal and post-menopausal conditions.

3. Results and Discussion

After comparing the mean absorbances between all eight conditions, the results of the ordinary one-way ANOVA, $F(7,16) = 35.28$, $p<0.0001$, $R^2 = 0.939$, demonstrate that hormone treatment had a significant effect on lipid accumulation overall. The large F-value illustrates that the means differ highly, with little probability of being due to random variation, and easily attributable to the different hormone treatments. The ANOVA test results are supported by the Brown-Forsythe test that resulted in $F(7,16) = 0.7556$, p-value = 0.6310; since $p>0.05$, there is no significant difference in variance across the groups.

3.1) The role of estrogen: High estrogen alone resulted in the clearest suppression of lipid accumulation in all eight groups, i.e., the value of mean absorbance of Oil Red O from the high estrogen sample was highest (Table A, Appendix). Tukey's multiple comparisons test revealed a highly significant, large difference in mean absorbance between high estrogen and the control, as well as the low estrogen condition as seen in Figure 4. The findings are also summarized in Table 2.

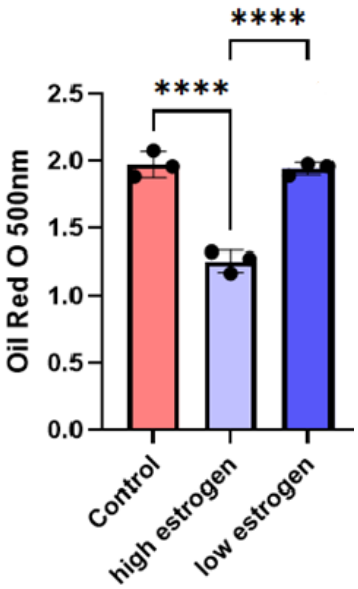

Fig 4: Bar chart showing mean absorbances of control, high estrogen and low estrogen conditions
The figure shows that control and low estrogen have high mean absorbances with overlapping error bars (no significant difference between means). The mean absorbance produced by high estrogen condition is significantly low.

Comparison	Mean difference	95% CI of mean diff.	Significance
Control vs. High estrogen	0.7170	0.4624 to 0.9716	****
Control vs. Low estrogen	0.03067	-0.2239 to 0.2852	ns
High estrogen vs. Low estrogen	-0.6863	-0.9409 to -0.4318	****

Table 2: Results of comparing mean absorbances of pairs solely involving estrogen
Note. *The difference is not significant when the 95% CI passes through zero.*
***** means p < .0001. ns = not significant.*

As lipid accumulation in the adipocytes decreased significantly under the high estrogen condition relative to the control, it is possible that lipid metabolism was promoted *or* lipid droplet formation was impeded by the presence of estrogen. This study aligns with the observations of lipolytic effects of estrogen in literature from Yoon (2009) and Zhu et al. (2013). However, at the low concentration of estrogen aligned with postmenopausal condition, the lipid accumulation had no significant difference with control, which indicates that estrogen concentration beyond a certain threshold is required for any noticeable change in lipid profile. Thus, the average postmenopausal concentration seems to be insufficient for the presence of estrogen to promote reduced lipid accumulation.

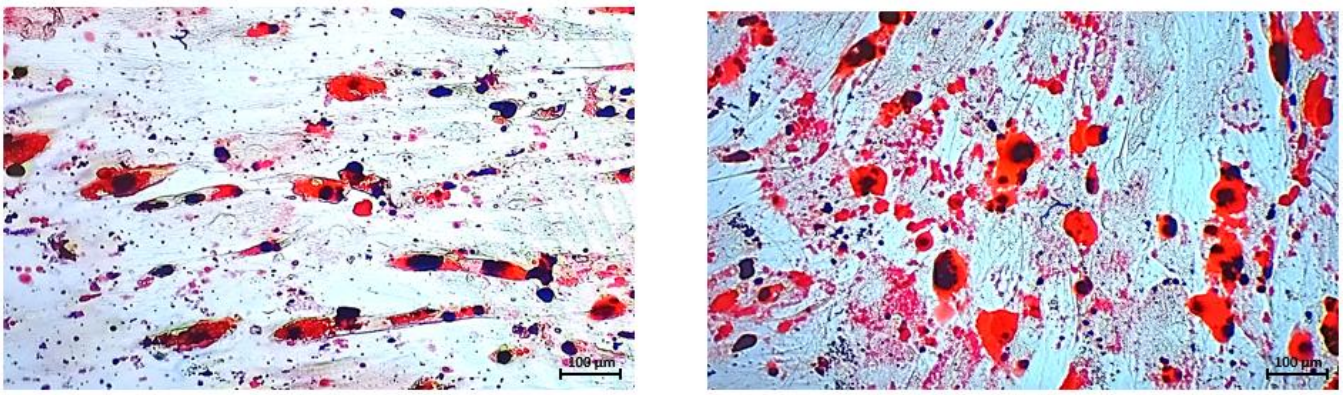

Fig 5: Microscope images of Oil Red O-stained high estrogen treated adipocytes (left) and low estrogen treated adipocytes (right)
The images at 20x magnification show lipid droplets stained red inside the adipocytes. Adipocytes in the high estrogen condition visibly have less lipid accumulation in small, isolated and scattered clusters of lipid droplets. The low estrogen condition produced high lipid accumulation in large, dense clusters with widespread distribution.

3.2) The role of progesterone: Comparing conditions involving progesterone led to nuanced findings. Table 3 shows the results - lipid accumulation was found to be less than control upon treating adipocytes solely with

progesterone of both concentrations, but the concentration did not produce significant difference in the lipid profiles (as the mean difference between high progesterone and low progesterone conditions is not significant). Regardless, the effect was significantly lower compared to that of estrogen.

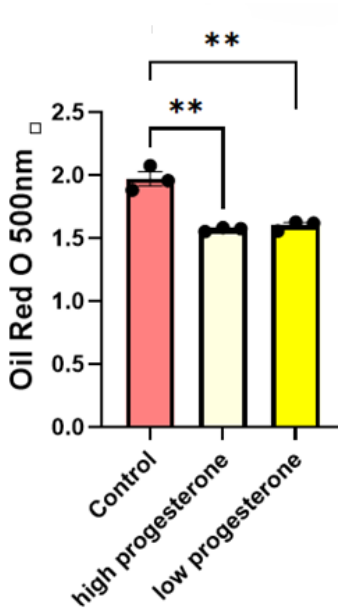

Fig 6: Bar chart showing mean absorbances of control, high progesterone and low progesterone conditions
The figure shows that both progesterone conditions produced lower mean absorbances than control.

Comparison	Mean difference	95% CI of mean diff.	Significance
Control vs. High progesterone	0.4013	0.1468 to 0.6559	**
Control vs. Low progesterone	0.3707	0.1161 to 0.6252	**
High progesterone vs. Low progesterone	-0.03067	-0.2852 to 0.2239	ns
High estrogen vs. High progesterone	-0.3157	-0.5702 to -0.06109	*

Table 3: Results of comparing mean absorbances of pairs solely involving progesterone
***Note**. * means p<0.05, ** means p<0.01, ns = not significant.*

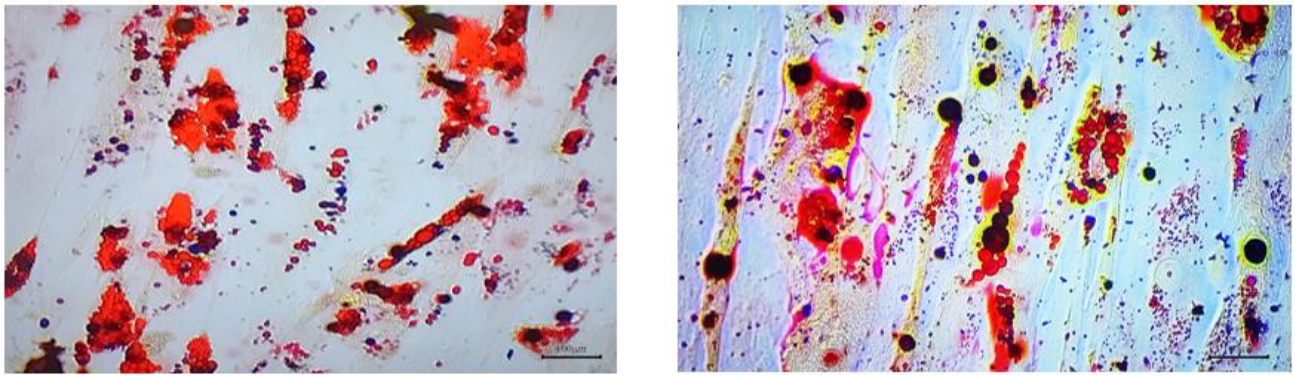

Fig 7: Microscope images of Oil Red O-stained high progesterone treated adipocytes (left) and low progesterone treated adipocytes (right)
The images at 20x magnification show lipid droplets stained red inside the adipocytes. Under both conditions, the adipocytes produced clusters of lipid droplets, some of which are notably large. The clusters appear larger in the high progesterone treated cells.

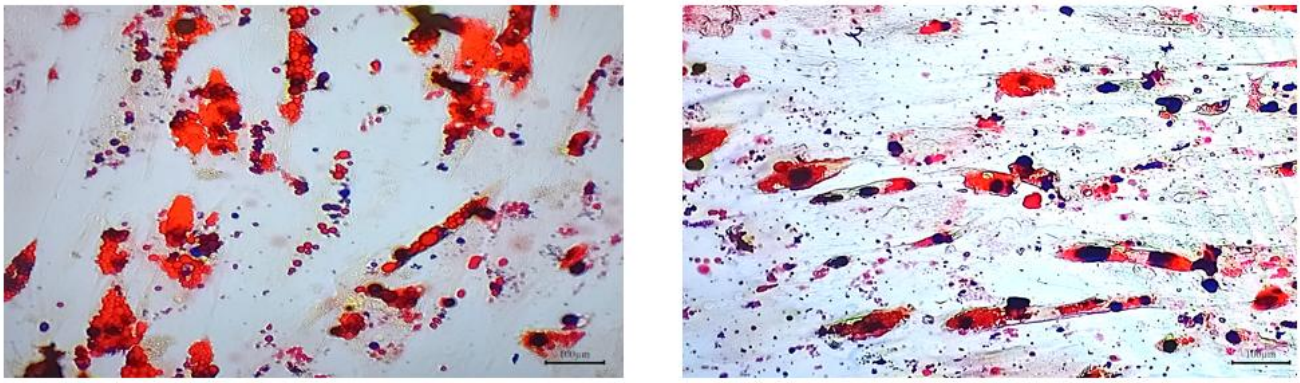

Fig 8: Microscope images of Oil Red O-stained high progesterone treated adipocytes (left) and high estrogen treated adipocytes (right)
The images at 20x magnification show lipid droplets stained red inside the adipocytes. Under the high progesterone condition, there are denser clusters of lipid with larger number of droplets compared to adipocytes in the high estrogen condition.

However, the role of progesterone cannot be assumed to be lipolytic with conviction through looking at this data only. Figure 7 shows how despite having similar lipid quantity under the two conditions, the adipocytes produced visibly larger clusters of lipid droplets under high progesterone concentration. Moreover, delving into the interaction of progesterone with estrogen in the combined treatment (which better reflects physiological reality of their hormonal effect in the body) reveals that it may counteract estrogen's potent role in suppressing lipid accumulation. The lipid accumulation was larger when treated in high estrogen and progesterone concentrations combined, compared to treating the adipocytes in high estrogen only. This is clearly shown by the significance of the mean difference of the two groups shown in Table 4.

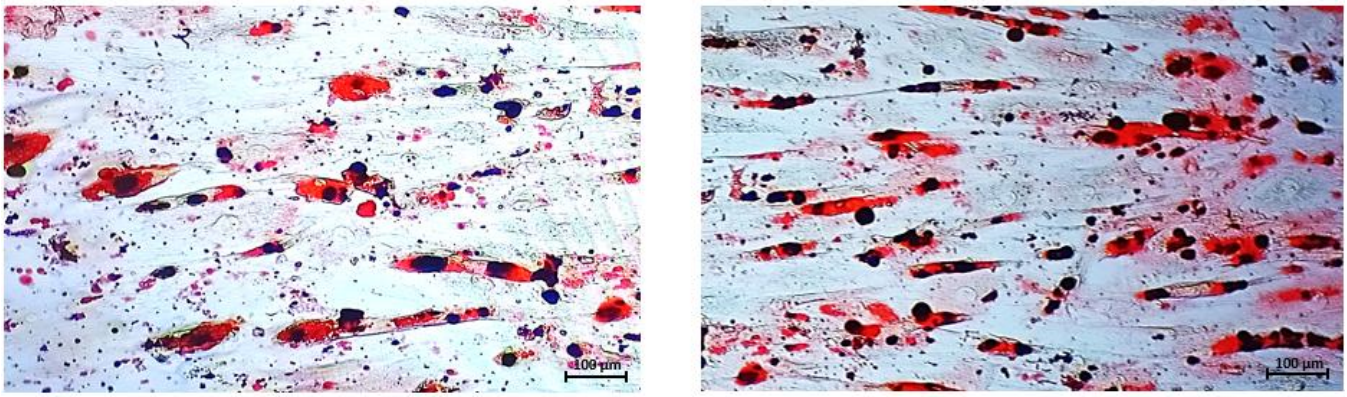

Fig 9: Microscope images of Oil Red O-stained high estrogen treated adipocytes (left) and high estrogen and progesterone treated adipocytes (right)

The images at 20x magnification show lipid droplets stained red inside the adipocytes. The shape of the lipid clusters and appearance of the droplets look similar in both groups of adipocytes, but the clusters are more abundant and widespread in the second group.

Comparison	Mean difference	95% CI of mean difference	Significance
High estrogen vs. High estrogen + progesterone	-0.2670	-0.5216 to -0.01243	*

Table 4: Result of comparing mean absorbances of high estrogen vs. high estrogen and progesterone combination
Note. ** means $p<0.05$*

Overall, our results showed evidence of progesterone both promoting and hindering lipid suppression in two different samples – leading to ambiguity regarding the role of progesterone.

3.3) The overall comparison of premenopausal condition vs postmenopausal condition: To answer the main research question, Tukey's multiple comparison test results for the combined hormonal condition pairs, reflecting mid-luteal phase serum concentrations and postmenopausal serum concentrations, are relevant. The high estrogen and progesterone (premenopausal) condition resulted in a significantly lower mean absorbance compared to low estrogen and progesterone (postmenopausal) condition (Table 5).

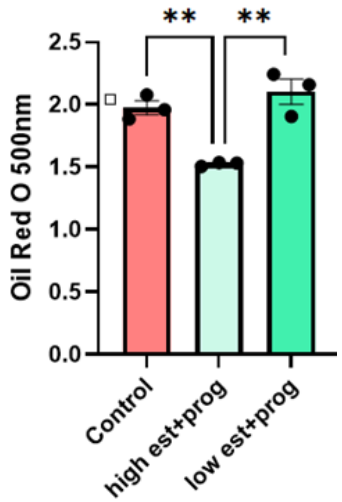

Fig 10: Bar chart showing mean absorbances of control, premenopausal condition, postmenopausal condition
The figure shows that the premenopausal condition (high est + prog) produced significantly lower mean absorbance than the postmenopausal condition (low est + prog), which has overlapping error bar with the control.

Comparison	Mean difference	95% CI of mean difference	Significance
High estrogen + progesterone vs low estrogen + progesterone	-0.5783	-0.8329 to -0.3238	****

Table 5: Result of comparing mean absorbances of premenopausal and postmenopausal condition groups
Note. ***** means p<0.0001.*

The postmenopausal hormonal environment significantly promotes more lipid accumulation in adipocytes relative to the premenopausal environment. Lipid accumulation is significantly suppressed by the combined hormonal condition despite the slight lipolysis-countering effect of progesterone observed earlier (Section 3.2, Table 4). This provides direct cellular-level evidence regarding the premise of postmenopausal obesity outlined in the introduction, which was previously only known from observational studies.

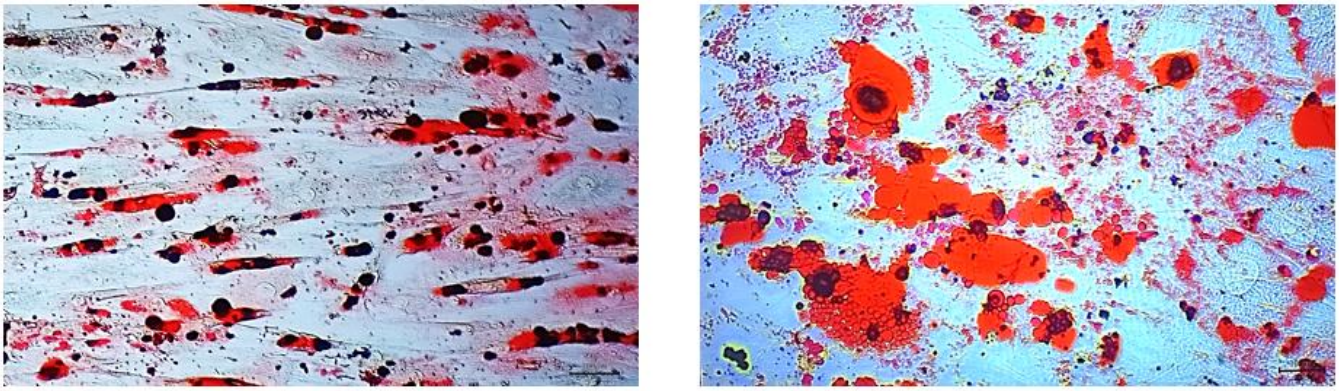

Fig 11: Microscope images of Oil Red O-stained *high estrogen and progesterone* treated adipocytes (left) and *low estrogen and progesterone* treated adipocytes (right)
The images at 20x magnification show lipid droplets stained red inside the adipocytes. The image on the left show smaller clusters with few lipid droplets dispersed on the field. The image on the right shows a contrasting field with widespread staining, featuring large, rounded clusters densely packed with droplets of different sizes.

It is notable that the postmenopausal condition produced the highest mean absorbance of all the groups, and had lipid accumulation similar to the control (Table A, Appendix).

Comparison	Mean difference	95% CI of mean difference	Significance
Control vs. low estrogen + progesterone	-0.1283	-0.3829 to 0.1262	ns

Table 6: Result of comparing mean absorbances of control and postmenopausal condition group
The difference between the absorbance means is very low and insignificant, since the 95% CI passes through zero.
Note. *ns = not significant*

The result indicates that without estrogen and progesterone working at meaningful concentrations as physiologically co-secreted in menstruating women, the adipocytes default to the baseline lipid accumulation that occurs without any hormonal activity at all. Thus, the low serum concentrations of the sex hormones after menstrual transition must lead to such unregulated lipid accumulation in adipocytes in the body, as well.

3.4) The action of HRT: Since the combined role of the two hormones demonstrated highly effective regulatory action on lipid accumulation, drawing a contrasting parallel with the postmenopausal state (while investigating each hormone's role in isolation left some unclarity), it created a greater question about the efficacy of HRT - to learn whether it can produce the same effect on lipid metabolism despite producing lower serum concentrations of the hormones than premenopausal levels (as shown in Table 1). For reference, the HRT condition in this experiment

was aligned with serum concentrations resulting from TX-001HR of doses 1 mg estradiol and 100 mg progesterone, found in widely available oral HRT pill, Bijuva (Lobo, 2019).

The HRT condition resulted in lipid accumulation that is statistically similar to high estrogen in isolation and the premenopausal condition (which are both groups showing high suppression of lipid accumulation). Moreover, the lipid accumulation is significantly lower than the control and postmenopausal state. These comparisons are made apparent in Figure 12.

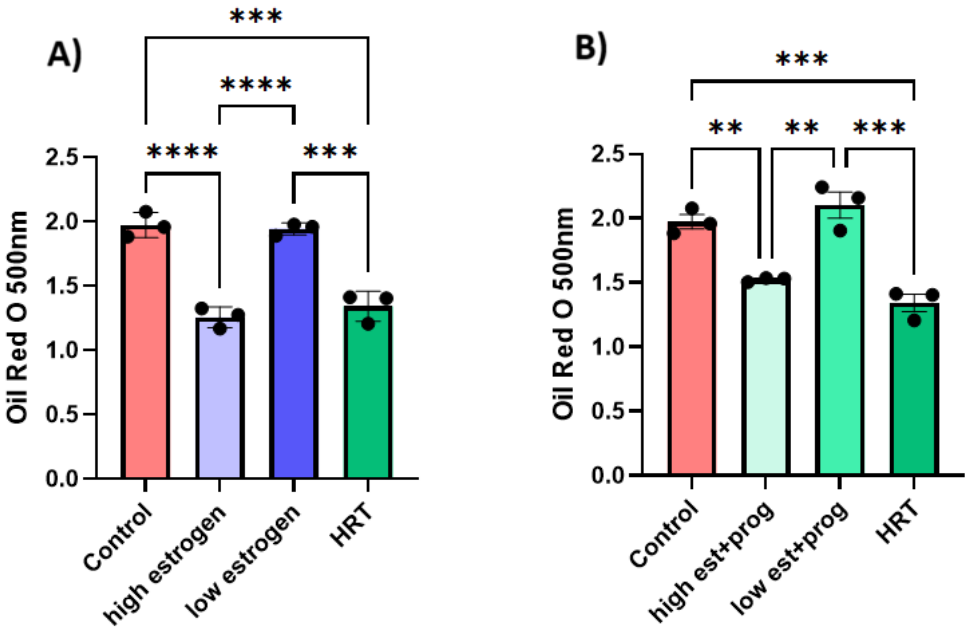

Fig 12: Bar charts comparing mean absorbances of relevant conditions with HRT
This figure shows that the mean absorbance of HRT mimic is significantly lower than the low estrogen condition and baseline control in A) as well as postmenopausal (high est+prog) condition in B). The error bar for HRT mimic also overlaps with high estrogen in A).

Comparison	Mean difference	95% CI of mean difference	Significance
Control vs. HRT	0.6327	0.3781 to 0.8872	****
High est+prog vs. HRT	0.1827	-0.07191 to 0.4372	ns
Low est+prog vs. HRT	0.7610	0.5064 to 1.016	****
High estrogen vs. HRT	-0.08433	-0.3389 to 0.1702	ns

Table 7: Result of comparing pairs of mean absorbances involving HRT mimic
Note. ***** means p<0.0001, ns = not significant*

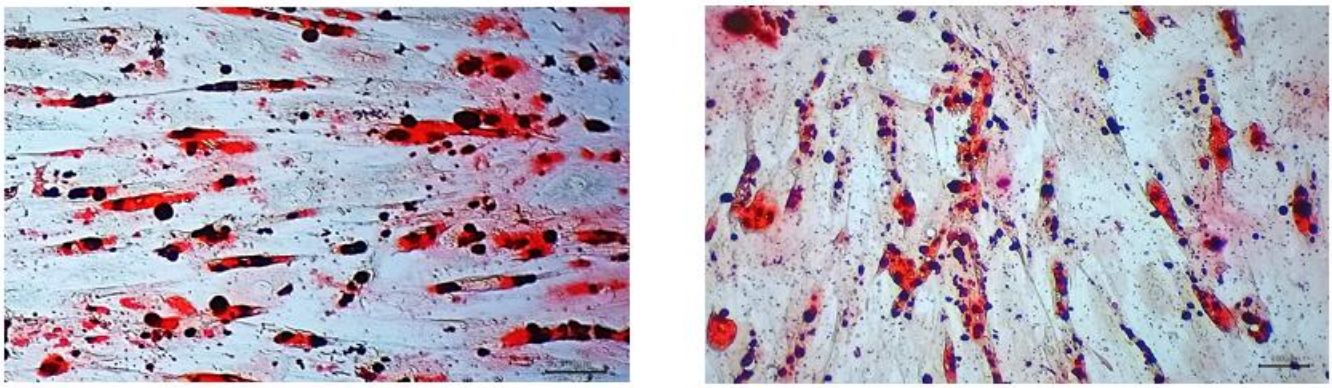

Fig 13: Microscope images of Oil Red O-stained *high estrogen and progesterone* treated adipocytes (left) and HRT treated adipocytes (right)
The images at 20x magnification show lipid droplets stained red inside the adipocytes. The clusters of lipid droplets in the HRT group look visibly similar to the premenopausal condition – similar shape with few lipid droplets and minimal staining.

Since the HRT treatment (which used realistic hormone concentrations aligned with a popular market-available oral pill) was able to mimic a premenopausal state of adipocytes so strongly, our research backs the effectiveness of combined HRT pills similar to Bijuva in their protective role against augmented lipid accumulation. This finding also confirms that the lower serum concentrations from taking HRT is still highly effective to demonstrate the same level of lipid suppression as premenopausal state. As mentioned earlier, the presence of estrogen is required above a certain threshold to show any lipolytic effect – the serum concentration maintained by HRT is sufficiently beyond the threshold to suppress lipid accumulation despite being lower (unlike the postmenopausal concentration, which is too low to have a difference on lipid profile).

4. Limitations

The greatest limitation was the restricted time of this project, which did not allow opportunities to replicate the experimental procedure or correct any errors made in the execution. For instance, the Oil Red O absorbance readings were taken by taking one sample of eluded dye per well. There was not sufficient time to mend this shortcoming by doing it over with triplicates of each well in the microplate. The triplicates would allow us to confirm that the absorbance readings from each well are uniform. Secondly, despite making strong conclusions through the

significant results of the ANOVA test, the small sample size of n=3 well replicates per condition introduced uncertainty to the findings i.e., the wide confidence intervals.

The progesterone treatment accuracy was also limited, as the project used a synthetic progesterone, which is structurally different from the natural progesterone (P4) found in human bodies. This was due to wanting to mimic HRT but not add too many variables so opted to use the same synthetic progesterone for the high and low treatments as well.

Additionally, the cellular observations made in the study only provided data about the relative lipid accumulations at the endpoint without actually indicating the underlying mechanism. For example, it is unclear if lipid accumulation would have decreased due to higher lipolysis or lower lipogenesis. Lastly, in-vitro models naturally introduce a limitation of physiological accuracy as isolated cultured adipocytes may not perfectly reflect the behavior of cells inside the body, where they are influenced by many other factors.

5. Conclusion

Overall, this study accomplished its goal of extending existing literature and finding cellular-level pilot data that serves as evidence of the observed patterns of rising obesity in women after menopausal transition, and the associated increased risks of cardiometabolic diseases. It found estrogen to be the dominant driving factor of lipid suppression in premenopausal women, the postmenopausal decline of which leads to higher lipid quantity in adipocytes (ultimately leading to higher adiposity in the body). Progesterone in isolation suppresses lipid growth to some extent, but not at the same scale as estrogen. However, the interaction of progesterone also neutralizes the action of estrogen in the combined hormonal environment.

In any case, the combined decline of both hormones after menopause significantly increases lipid accumulation and can be deduced as the underlying cause of postmenopausal obesity. Lastly, the HRT condition provided mechanistic support for HRT's role as a treatment for postmenopausal obesity as a positive result on lipid suppression was observed from using real combined oral pill hormonal doses as a reference.

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Appendix

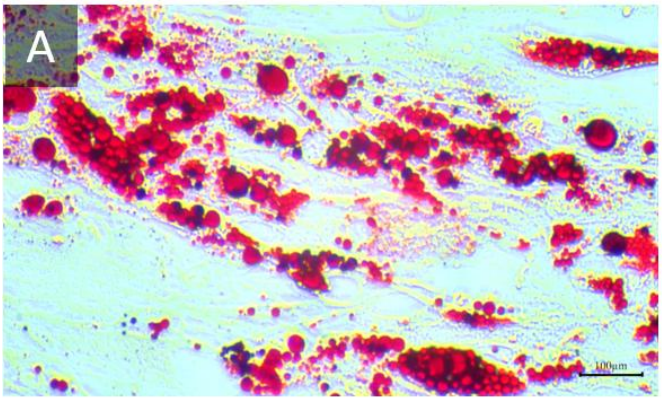
Table A: The absorbance readings of Oil Red O at 500 nm (raw data)

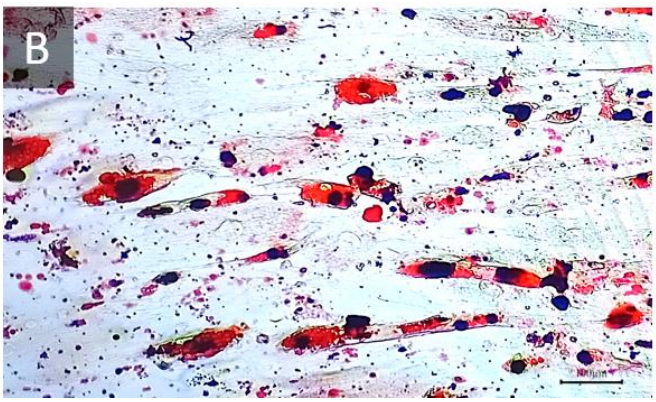
Control	High estrogen	Low estrogen	High progesterone	Low progesterone	High est+prog	Low est+prog	HRT
1.885	1.327	1.891	1.579	1.622	1.505	1.906	1.406
2.079	1.169	1.979	1.555	1.559	1.532	2.160	1.207
1.960	1.277	1.962	1.586	1.631	1.537	2.243	1.413
1.975	**1.258**	**1.944**	**1.573**	**1.604**	**1.525**	**2.103**	**1.342**

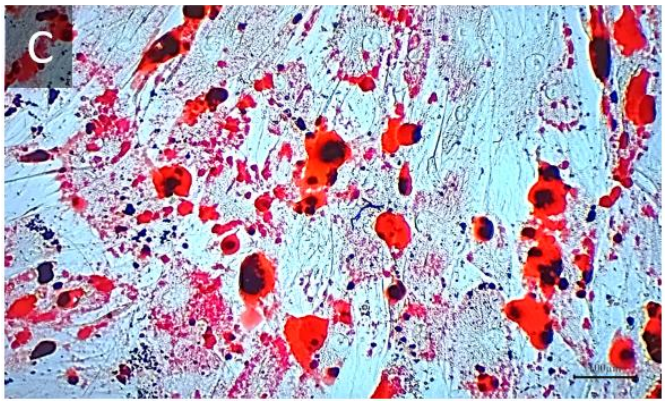
the mean absorbances are written in bold (last row)

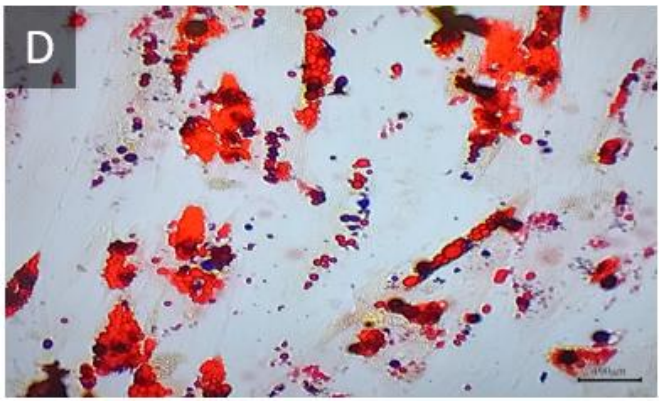
Fig A-H: Compiled microscope images of all Oil Red O-stained adipocyte samples
images are all under 20x magnification

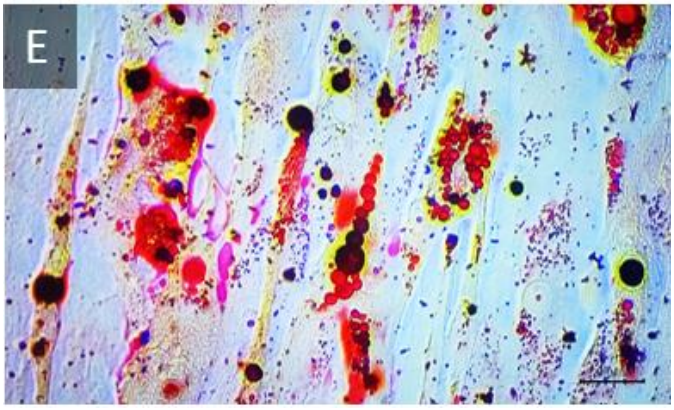
A – control
B – high estrogen
C – low estrogen
D – high progesterone
E – low progesterone
F – high estrogen and progesterone
G – low estrogen and progesterone
H – HRT mimic

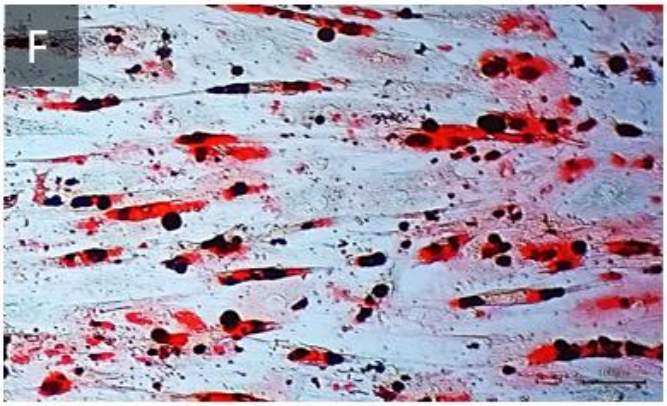


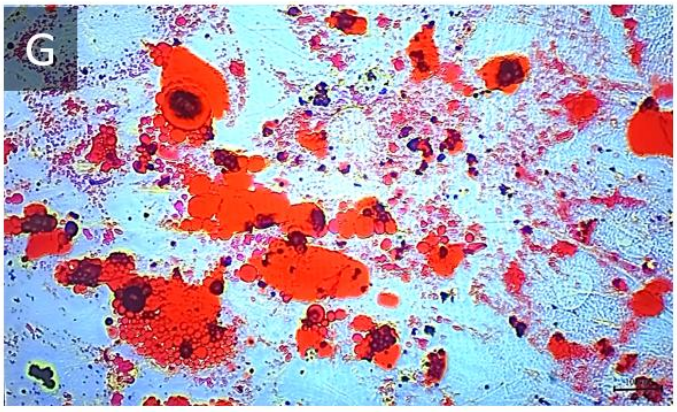


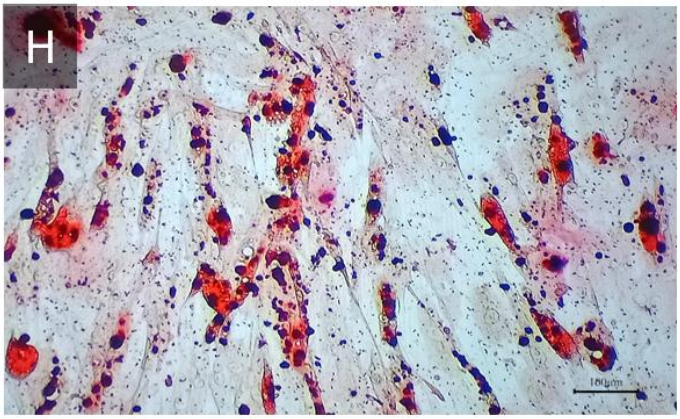

C


D


E


F


G


H