

Introduction and Background

- Ovarian cancer is the fifth most common cause of death in women.
- Understanding underlying mechanisms of ovarian cancer will help in developing better treatments.
- Recently, it has been found that ovarian cancer patients with poor prognosis have presented with elevated stress hormones.
- This study was designed to find out the metabolic changes in ovarian cancer cells (HeyA8) when treated with a stress hormone cortisol.
- To study this, NMR spectroscopy has been employed as an analytical tool.

Hypothesis: Ovarian cancer cells treated with stress hormones show altered metabolism.

NMR Metabolomics Flow Chart

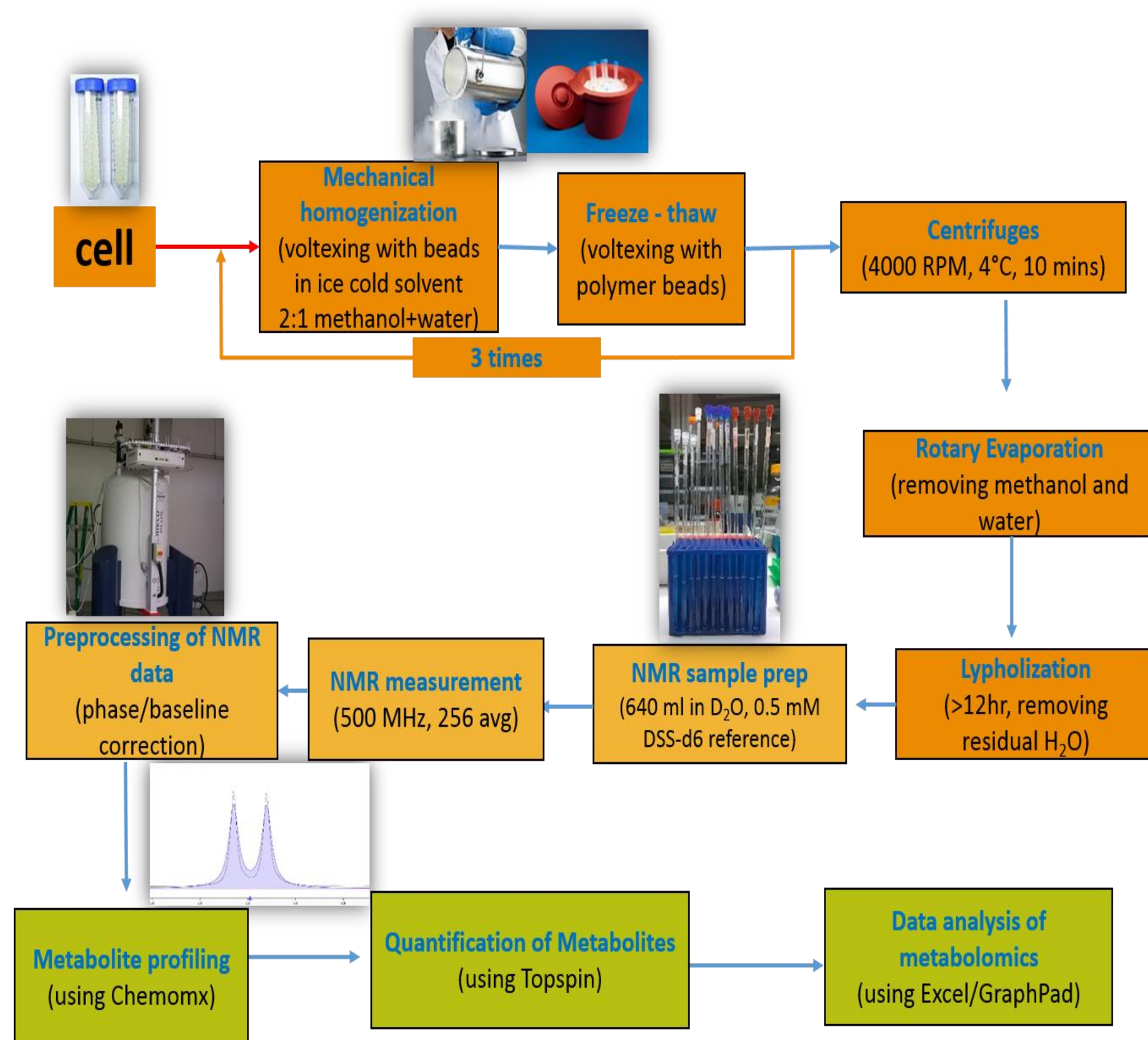


Figure 1. Flowchart for NMR Spectroscopy Based Metabolomics

Materials and Methods

- HeyA8 are high grade ovarian serous adenocarcinoma human cell lines used in this study.
- Metabolites are extracted from cancer cells using a methanol-water mixture, ceramic beads, three cycles of a mechanical homogenization and freeze-thawing process followed by centrifugation, rotary evaporation and lyophilization.
- The samples are prepared for nuclear magnetic resonance (NMR) spectroscopy by dissolving the sample in ²H₂O containing the reference compound 4,4-dimethyl-4-silapentane-1-sulfonic acid-d₆ (DSS).
- All the data was acquired on a Bruker NMR spectrometer operating at 500 MHz ¹H resonance equipped with a cryogenically cooled triple resonance (¹H, ¹³C, ¹⁵N) TXI probe.
- Identification of metabolite peaks was done through Chemomx and the Human Metabolomic Database (HMDB); finally, the peaks were integrated in Topspin and normalized to the reference compound (DSS). All 1-D proton NMR spectra were normalized to the cell count before analysis.

Results and Discussions

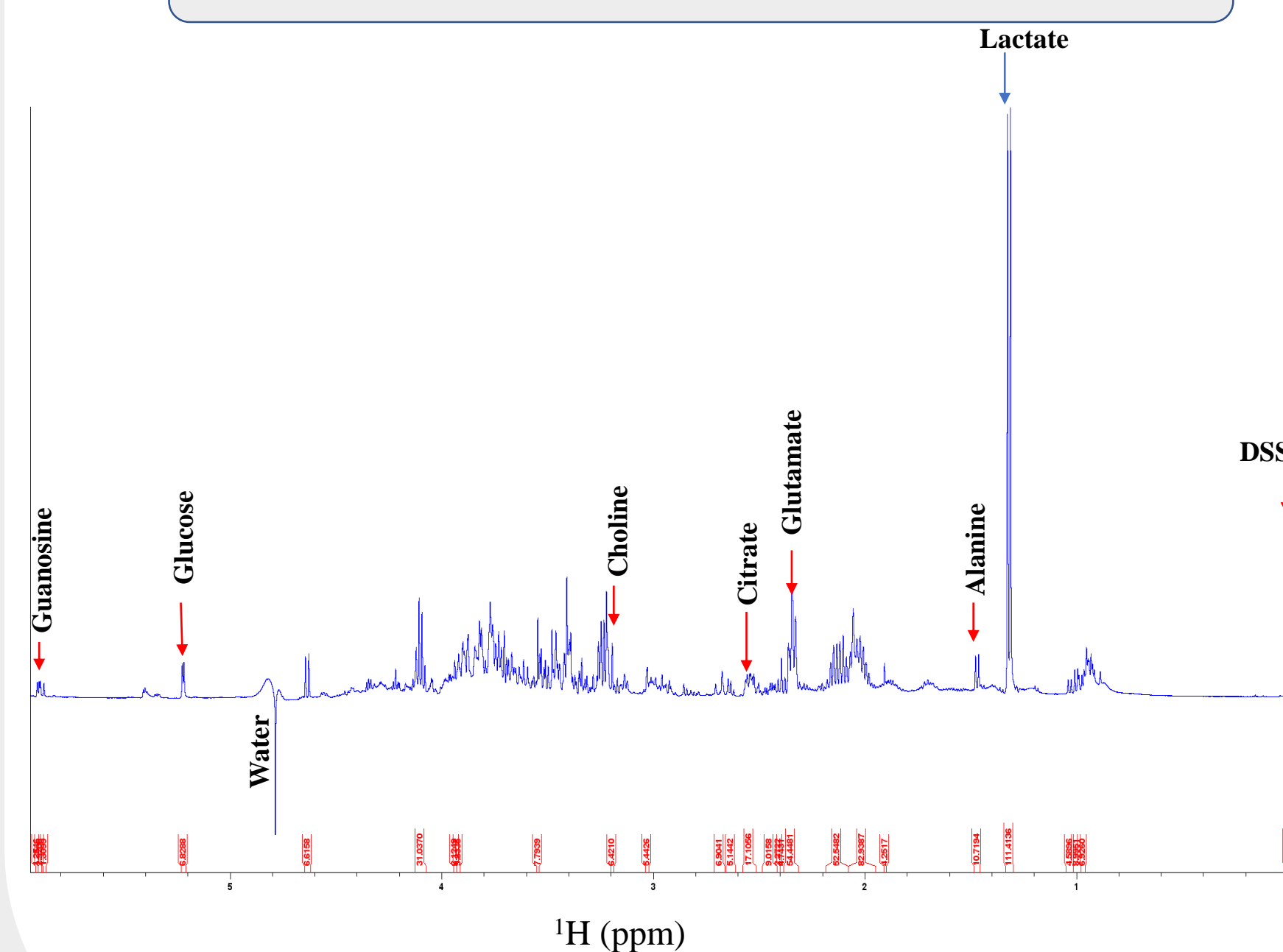


Figure 2: 1D ¹H NMR spectrum with assigned key metabolites

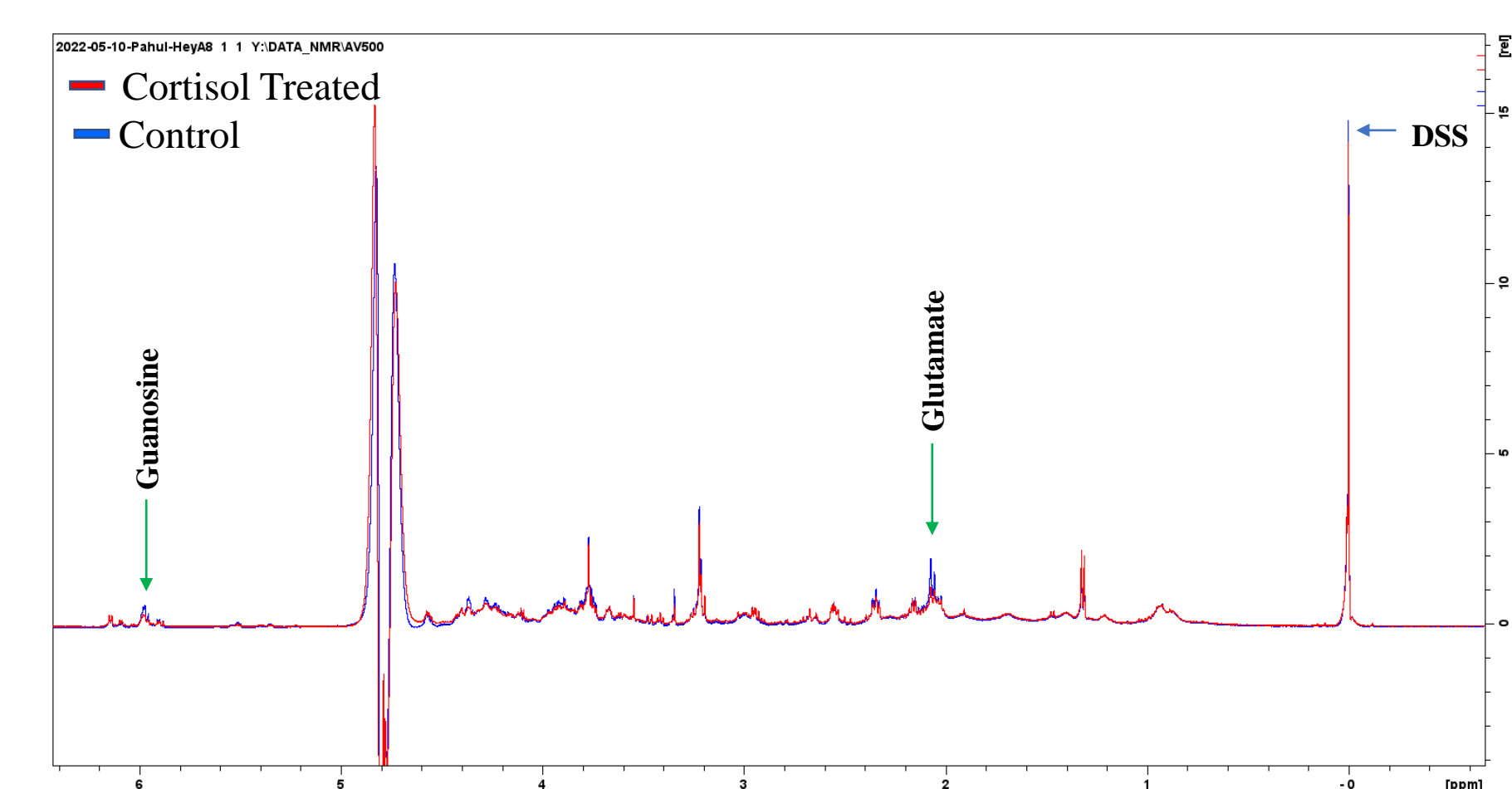


Figure 3: Metabolites altered significantly in cortisol treated cells are shown in the 1D ¹H spectrum

Conclusions

- Initial studies showed a difference in metabolites glutamate, guanosine and uridine.
- However, more samples need to be analyzed to obtain statistical significance.

Acknowledgements

I am grateful to Dr. Pratip Bhattacharya and the Department of Cancer Systems Imaging at MD Anderson for hosting me. I would especially like to thank Dr. Shivanand Pudakalakatti for guiding me through the process of metabolic profiling via NMR spectroscopy and helping me interpret the results. I would also like to extend thanks to the Sood Laboratory for providing the ovarian cancer cells for the study.

Funding Acknowledgement: Paytience Smith was supported by a generous gift from H-E-B directed to the Partnership in Cancer Science and Medicine Program, Office of Faculty Diversity, Equity and Inclusion.

References

1. Arora T, Mullangi S, Lekkala MR. Ovarian Cancer. [Updated 2022 Jan 4]. In: *StatPearls* [Internet]. Treasure Island (FL): StatPearls Publishing; 2022.
2. Schrepf A, Thaker PH, Goodheart MJ, Bender D, Slavich GM, et al. Diurnal cortisol and survival in epithelial ovarian cancer. *Psychoneuroendocrinology*. 2015; 53:256-67.
3. Pudakalakatti S, Titus M, Enriquez JS, Ramachandran S, et al. Identifying the Metabolic Signatures of PPARδ-Overexpressing Gastric Tumors. *Int J Mol Sci*. 2022; 23(3):1645.
4. Schrepf A, Clevenger L, Christensen D, DeGeest K, et al. Cortisol and inflammatory processes in ovarian cancer patients following primary treatment: relationships with depression, fatigue, and disability. *Brain Behav Immun*. 2013; 30: S126-34