Many women worldwide reported changes in their menstrual bleeding patterns after the first and second doses of the COVID-19 vaccine. Unfortunately, these menstrual abnormalities were only partially studied. In this article, the authors from Israel investigated the direct effect of the BNT162b2 mRNA COVID-19 vaccine on the mRNA expression of genes related to the activity of ovarian granulosa cells (GCs), which support the oocytes during folliculogenesis. They also examined the possible association between these findings and post-vaccination menstrual disorders.

The BNT162b2 mRNA COVID-19 vaccine (Pfizer-BioNTech) was the first vaccine authorized by the FDA. The vaccine utilizes a lipid nanoparticle (LNP) technology developed by Acuitas Therapeutics Inc. The authors emphasize that Acuitas Therapeutics Inc. in its "Final Report" to the FDA, monitored the distribution and accumulation of LNP envelope containing tagged-mRNA in the body of research-model rats and suggested that the ovaries were one of the four organs that accumulated the LNP vehicle.

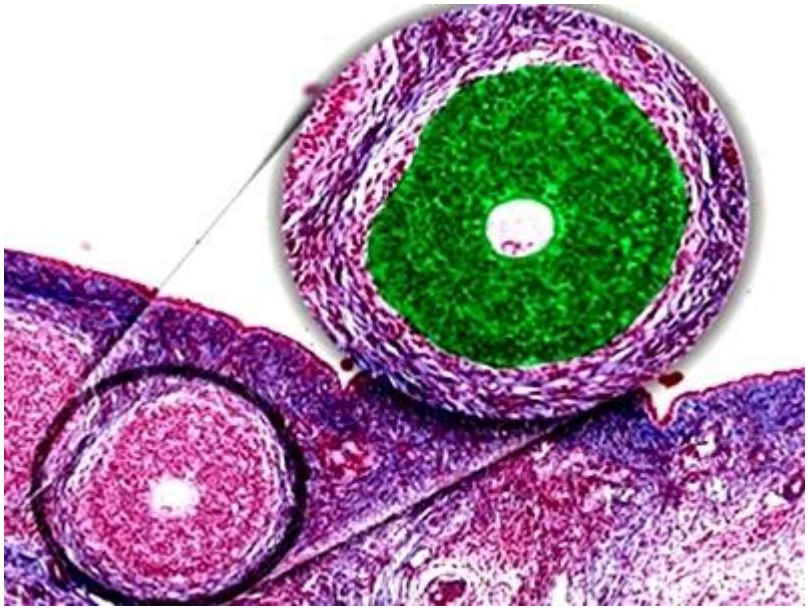
The menstrual cycle consists of follicular, ovulatory, and luteal phases. The follicular phase lasts from the start of menstrual bleeding until ovulation and is determined by follicle-stimulating hormone (FSH), which promotes follicle growth. The luteal phase lasts from ovulation to the next menstrual bleeding and is regulated by an increase in luteinizing hormone (LH), which promotes *corpus luteum* formation. The most important functional unit of the ovary is the follicle, composed of an oocyte surrounded by granulosa cells (GCs), which support the oocyte during folliculogenesis. Granulosa cells are present in all stages of follicular development except in the primordial follicle, which is surrounded by a single layer of follicular cells. The authors emphasized that GCs are endocrine cells that participate in the hypothalamic-pituitary-ovarian (HPO) axis. Stimulated by FSH, the GCs secrete endocrine and paracrine regulators, such as estrogens (produced within the GCs), anti-Mullerian hormone (AMH), and inhibins. These hormones, in turn, regulate FSH, directly by reducing its synthesis and secretion at the level of the hypophysis (estrogens and inhibins) or indirectly by reducing the sensitivity of follicles to FSH (AMH).

Anti-Mullerian hormone (AMH), produced exclusively by GCs, has a paracrine effect on other follicles. It down-regulates the FSH receptor (FSHR) level in pre-antral follicles and inhibits the activation of primordial follicles from the ovarian pool. Inhibin B is produced primarily by GCs of FSH-dependent growing antral follicles and secreted during the follicular phase of the menstrual cycle, before ovulation. Inhibin A is predominantly expressed by the *corpus luteum*.

As GCs play a significant role in the regulation of the HPO axis, and Acutias Therapeutics

Inc. in its “Final Report” to the FDA, reported the accumulation of LNP vehicles in ovaries, the authors hypothesized that the direct impact of the vaccine on GCs may alter the expression and secretion of ovarian follicular hormonal regulators. Consequently, this affects the menstrual cycle. They focused on inhibin B, which participates in the HPO feedback loop.

Interestingly, a previous animal study has shown that different phases of the menstrual cycle differently influence the accumulation and effectiveness of nanoparticles in the female reproductive organs. The maximal accumulation of 80nm liposomes loaded with gadolinium was found in the ovaries and uterus during estrus.
https://discovermednews.com/nanoparticles-accumulate-in-mouse-reproductive-system/



About the study

The authors exposed human primary ovarian granulosa cells (hpGCs) obtained from women 20–45 years of age undergoing IVF treatments, to two concentrations of the BNT162b2 mRNA vaccine (“injected dose” or “end-organ dose”) for 24 or 48 hours. According to the Final Report of Acuitas to the FDA, the end-organ dose was approximately 0.1% of the injected dose, representing the accumulated concentration in women’s ovaries. Non-treated

cells were control cells.

Vitality in harvested hpGCs exposed to the BNT162b2 mRNA vaccine for 24 or 48 hours was evaluated by the MTT assay. The mRNA expression of genes related to the activity of GCs was examined by quantitative polymerase chain reaction, including mRNA expression of aromatase, interleukin (IL)-8, AMH, FSHR, inhibin A, and inhibin B genes. The levels of inhibin B and inhibin A secreted from the hpGCs in the culture medium were evaluated by the ELISA test.

The researchers also investigated whether modifications in proteins related to GCs' activity could be detected in the blood of five women before and approximately one month after the third dose of the BNT162b2 mRNA vaccine. Additionally, 124 women were interviewed about changes in their menstrual cycle approximately 4 months after receiving the third dose of the BNT162b2 vaccine.

Results

After 24 or 48 hours of exposure to either the injected or the end-organ doses of the BNT162b2 vaccine, the viability of hpGCs was not compromised.

After 24 hours of exposure to the injected dose (a 1000 times higher dose than the end-organ dose) of the BNT162b2 vaccine, the results showed decreased mRNA levels of aromatase and FSHR and prominently increased mRNA levels of IL-8. The 24-hour exposure to the end-organ dose increased the mRNA level of inhibin B, but didn't reach statistical significance.

After 48 hours of exposure, both doses of the BNT162b2 vaccine decreased mRNA levels of AMH, suggesting a similar *in vivo* reduction of AMH expression in ovarian follicles after the vaccination. In addition, the end-organ dose increased the mRNA level of inhibin B (more than 200%) after 48 hours of exposure. Since both inhibin B and AMH are secreted from the GCs of growing follicles, the authors stated that these results may lead to a larger population of hormonally active follicles, a higher serum level of inhibin B, and disruption to the cycle.

After 48 hours of exposure to the end-organ dose of the BNT162b2 vaccine, the results showed an increase in inhibin B secreted from the hpGCs in the culture medium in three independent experiments, but statistically insignificant.

After exposure to both concentrations of the BNT162b2 mRNA vaccine ("injected dose" or

"end-organ dose") for 24 or 48 hours, the changes in inhibin A levels in the culture medium were not detected. It was expected, as inhibin A is expressed mainly by the *corpus luteum*.

The *in vivo* analysis of the ratio between FSH and inhibin B levels revealed its change by 2–3 fold in five women before and approximately one month after the third dose of the COVID vaccine. These women also reported changes in menstrual bleeding patterns. According to the authors, the FSH/inhibin B ratio is relatively stable and independent of the day of the menstrual cycle. Also, every woman has her own FSH/inhibin B ratio, which remains relatively constant throughout her menstrual cycle. Accordingly, it seems that vaccination causes an immediate increase in inhibin B expression, which alters the menstrual cycle length and bleeding.

Out of 124 women who were interviewed, 40% of those with regular menstruation and 53% of those with irregular menstruation reported various changes in the menstrual cycle length and bleeding pattern after the third vaccination with BNT162b2.

Conclusion

The authors concluded that this study revealed that the BNT162b2 mRNA COVID-19 vaccine directly affects ovarian granulosa cells (GCs). This is a unique mechanism that could cause vaccine-related menstrual abnormalities. The cell vitality was not impaired, but their exposure to the end-organ concentration of the vaccine altered their activity, increasing mRNA expression of the inhibin B gene and decreasing mRNA expression of the AMH gene.

According to the authors, these changes could significantly affect FSH serum levels and the HPO axis in vaccinated women. This could lead to a disruption of follicular growth (i.e., too many follicles grow at the "wrong" time of the cycle) and activity (i.e., estrogen production), ultimately affecting the uterine cycle. A 2–3 fold change in the ratio between FSH and inhibin B protein levels found in the serum of women with menstrual disorders after the third dose of the COVID vaccine supports these findings.

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