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.

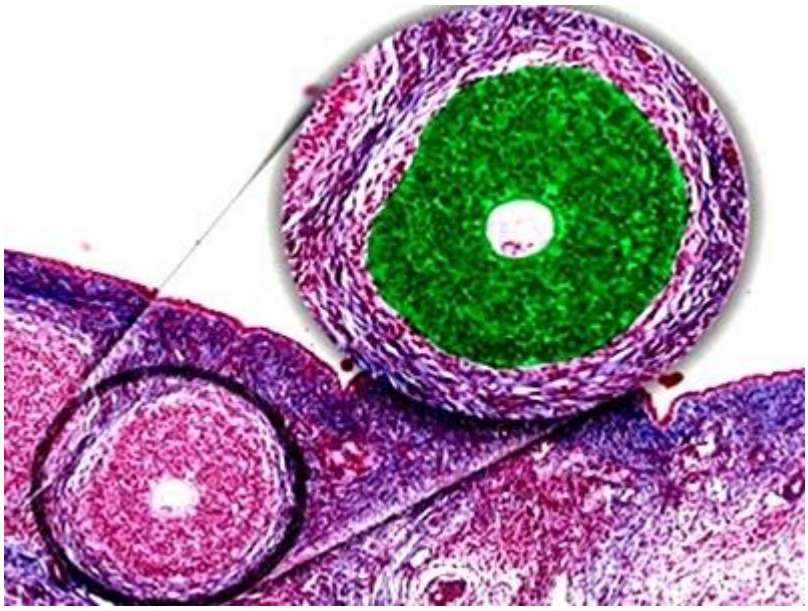
The menstrual cycle consists of follicular, ovulatory, and luteal phases. The follicular phase lasts from the start of menstrual bleeding until ovulation and is determined by follicle-stimulating hormone (FSH), which promotes follicle growth. The luteal phase lasts from ovulation to the next menstrual 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 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, one previous animal study has shown that 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. Furthermore, the viability of the pups in the doxorubicin-loaded liposomes group significantly decreased to 60%, indicating elevated ovarian toxicity of doxorubicin-loaded liposomes compared to free-doxorubicin.
https://discovermednews.com/nanoparticles-accumulate-in-mouse-reproductive-system/



About the study

The authors exposed human primary GCs (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. Control cells were non-treated 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 the 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 ELISA test.

The researchers also investigated whether *in vitro* 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. Also, 124 women were interviewed about the 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 of the vaccine increased the mRNA level of inhibin B but didn't reach statistical significance.

According to the "Final Report" of Acuitas Therapeutics Inc., the researchers continued to monitor the hpGCs exposed to the vaccine for 48 hours. At this time point, the alterations observed after the 24-hour exposure to the injected dose reverted to their baseline state. These findings indicate that the activity and responsiveness of human primary GCs were recovered.

However, both doses of the BNT162b2 vaccine decreased mRNA levels of AMH after 48 hours of exposure, suggesting a similar *in vivo* reduction of AMH expression in ovary follicles after the vaccination. In addition, the end-organ dose significantly increased (more

than 200%) inhibin B mRNA level after 48 hours of exposure. Since both inhibin B and AMH are secreted from the GCs of growing follicles, the authors stated that these findings may lead to a larger population of hormonally active follicles, a higher serum level of inhibin B, and disruption to the cycle.

After 48 h 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. The changes in inhibin A levels in the culture medium were not detected after exposure to both concentrations of the BNT162b2 mRNA vaccine ("injected dose" or "end-organ dose") for 24 or 48 hours. 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 COVID vaccine. In addition, these women reported changes in menstrual bleeding patterns. The authors noted that the FSH/InhibinB ratio is relatively stable, 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. According to the authors, these findings support their hypothesis that vaccination causes an immediate elevation in inhibin B expression, which leads to changes in the length of the menstrual cycle and bleeding.

Out of 124 women who were interviewed, 40% of those with regular menstruation and 53% of those with irregular menstruation, reported a variety of changes in the length of the menstrual cycle 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 GCs. This is a unique, independent mechanism for vaccine-related menstrual abnormalities. These findings were not caused by impaired cell vitality. The exposure to the end-organ concentration of the vaccine changed the activity of GCs and transcripts of two ovarian-regulatory key factors, prominently increasing inhibin B and decreasing AMH.

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 uterus 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 COVID vaccine, supports these findings.

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