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RESEARCH

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Phosphate concentrations in follicular fluid during assisted reproductive treatment: relevance for ovarian function and fertility outcomes

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Abstract

Background The role of follicular fluid phosphate for reproductive health and oocyte maturation is unclear. This study investigates the relationship between follicular fluid vs serum phosphate concentrations and the possible link with sex steroids during in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) at a Danish fertility clinic.

Methods A prospective cohort of infertile women who attended Danfert Fertility clinic (Copenhagen, Denmark) and received IVF or ICSI treatment between June 2015 and February 2017. Correlation analyses were performed with Spearman's Rank or Pearson's correlation, while categorical variables were analyzed with Chi-squared test.

Results In total, 110 participants were included in the study, and 33 of these achieved a live birth. Phosphate concentrations were higher in the follicular fluid compared to corresponding serum samples (1.16 mmol/L vs. 1.06 mmol/L, $p=0.002$) and there was a positive correlation between serum and follicular fluid phosphate concentrations ($r=0.43$, $p=0.007$). A positive trend was also found for calcium concentrations, though not statistically significant ($r=0.31$, $p=0.060$). Correlation analysis also showed a positive correlation between concentrations of phosphate and calcium in follicular fluid ($r=0.41$, $p<0.001$). A positive correlation was observed between concentrations of phosphate and testosterone in follicular fluid ($r=0.34$, $p<0.001$). When stratified into tertiles, we found no significant differences between live birth rates in follicular fluid phosphate ($p=0.624$), calcium ($p=0.207$), or testosterone ($p=0.841$).

Conclusions This study found that follicular fluid phosphate concentrations are higher than serum phosphate concentrations, suggesting possible local regulation. However, no significant association was found between follicular phosphate and ART outcomes. Further research is needed to explore its potential role in reproductive physiology.

Clinical trial Clinicaltrials.gov (NCT02437578; registration date 2015/04/16).

Keywords Infertility, Phosphate, Follicular fluid, Live birth rates

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Introduction

The growing demand for fertility treatments underscores an urgent need to identify reliable biomarkers for predicting assisted reproductive technology (ART) outcomes [1]. Such advancements aim to enhance treatment success rates while alleviating the significant burden on patients and healthcare system. Infertility, defined as the inability to conceive after 12 months of regular and unprotected intercourse, affects 7 to 26% of couples of the reproductive age worldwide [2, 3]. While much attention has been paid to factors such as serum hormone concentrations and oocyte morphology, the role of follicular fluid, a complex medium surrounding the oocyte, is still underexplored. Given its unique composition of hormones and minerals, follicular fluid may hold critical insights into reproductive potential.

Androgens, synthesized in the ovaries, play a pivotal role in the follicle. These hormones serve as precursors for estrogen production through aromatization in granulosa cells. Estradiol is vital for regulating the menstrual cycle, follicular development, and ovulation [4]. Androgens promote follicle growth and survival via androgen receptors and enhance granulosa cell sensitivity to follicle-stimulating hormone (FSH), crucial for maintaining antral follicles and ovulatory potential. Further highlighting the integral role of androgen-estradiol interplay in reproductive success [5].

The tightly regulated environment in the follicle also includes minerals such as phosphate and calcium, which are critical for numerous physiological processes. The systemic regulation of phosphate and calcium concentrations is a dynamic process controlled primarily by parathyroid hormone (PTH), fibroblast growth factor 23 (FGF23), and vitamin D, each of which modulates the balance of these minerals in bones, kidneys, and intestines [6, 7]. PTH, secreted by the parathyroid glands in response to low serum calcium concentrations, mobilizes phosphate and calcium from bone stores by stimulating bone resorption. Thus, serum calcium and phosphate increase, though PTH simultaneously promotes the excretion of phosphate by the kidneys to prevent hyperphosphatemia. PTH also increases renal calcium reabsorption and the conversion of vitamin D into its active form, 1,25-dihydroxyvitamin D (calcitriol) by enhancing renal CYP27B1 activity [8–10]. Conversely, FGF23, counteracts hyperphosphatemia by reducing renal phosphate reabsorption and active vitamin D synthesis [7, 11, 12]. PTH, FGF23, and vitamin D ensure stable serum calcium and phosphate levels, essential for skeletal health and cellular integrity across organ systems [13].

The role of minerals and their regulators in reproduction has been highlighted in recent years, mainly in men, where mineral concentrations have been positively associated with sperm quality [14, 15]. Phosphate has been shown to be one of these minerals, and the NaPi-IIb transporter has been found in the epididymis [16]. While it hasn't been studied whether the female possesses an equivalent, we speculate that a transporter similar to NaPi-IIb could also be present in the female reproductive system. However, phosphate also plays a critical role in fetal development [17]. High concentrations of phosphate in amniotic fluid during early gestation support essential processes like the nervous system and skeletal development. However, as pregnancy advances, amniotic fluid phosphate concentrations decrease. Lower-than-expected concentrations in the second trimester have been linked to preterm birth, suggesting that phosphate may serve as an indicator of both gestational progress and fetal health [18]. Despite phosphate's well-established significance in other areas of reproductive and fetal health, its role within the female follicular environment remains largely uncharted.

To our knowledge, the composition of follicular fluid, particularly concerning phosphate homeostasis, remains inadequately studied. Therefore, this study aims to investigate correlations between serum and follicular fluid phosphate concentrations, and whether phosphate concentrations impact pregnancy outcomes following ART.

Materials and methods

Study design and participants

The study included women from infertile couples who, together with their partners, pursued treatment at Danfert Fertility, a private fertility clinic in Denmark. All women included in the study underwent either in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) between June 2015 and February 2017. Blood samples were collected either before or on the day of oocyte aspiration. Three senior fertility specialists at Danfert Fertility were responsible for documenting the clinical data, treatment outcomes, and ICD-10 diagnoses associated with infertility for each couple. The study was conducted in accordance with the second Helsinki Declaration. This study received approval from the Regional Ethical Committee (reference H-15000931), and informed consent was obtained from all participants. Details of the study have been registered publicly under clinical trial identifier NCT02437578 (registration date 2015/04/16).

Sample collection

Blood samples were collected from participants prior to oocyte retrieval and stored at −20 °C. Sensitivity analysis showed no difference in measurement from those who had a blood sample on the same day or days prior to oocyte retrieval. Follicular fluid samples were obtained during oocyte retrieval from large follicles measuring

17–18 mm up to 23 mm. An initial 10 mL of follicular fluid was aspirated without flushing and pooled for each participant. Pooling of follicular fluid was done to ensure sufficient material for biochemical analysis. Samples with visible blood contamination were excluded. The pooled follicular fluid was centrifuged for 10 min at 3000 rpm, after which the supernatant was transferred to a new tube and stored at −20 °C until analysis.

Hormone measurements

Testosterone was analyzed using chemiluminescent immunoassays (Access, Beckman Coulter, USA), with detection limits of 0.35 nmol/L, and coefficient of variation (CV) of < 11%. Phosphate and calcium concentrations were measured using the Cobas system (Roche Diagnostics A/S, Hvidovre, Denmark) with CVs of 6% and 2.5%, respectively.

Statistical analysis

For baseline characteristics (Table 1) we included the samples from either the women's first visit or the visit where they achieved a pregnancy leading to a live birth. Continuous data were reported as the mean ± standard deviation (SD) when data was normally distributed or as median with interquartile range (25 th and 75 th percentiles) when it was not normally distributed. Data was inspected for normal distribution by histograms and QQ-plots. Categorical variables were expressed as counts and percentages. Differences between group means were analyzed using Student's t-test, and presented with a 95% Confidence interval. Differences between group medians were evaluated with the Mann–Whitney U test. Categorical variables were analyzed with the Chi-square test. A linear mixed model was used to analyze the impact of each subsequent visit on follicular fluid concentrations. Here, all visits for every participant were included. Paired t-tests were used to compare phosphate concentrations between serum and follicular fluid samples, as well as to analyze subgroup differences. Correlation analyses were conducted using Pearson's correlation when data was normally distributed, and Spearman's non-parametric analysis when data was not. Both were visualized through simple linear regression. All statistical analyses were conducted using R version 4.4.1 (R Core Team 2021. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org), with statistical significance set at $p < 0.05$.

Results

Baseline characteristics

The flow of the study is shown in Fig. 1A. 289 women were referred for ART at the fertility clinic. We excluded 146 women who only underwent intrauterine insemination (IUI) and 33 who did not have a follicular phosphate measurement. A total of 110 participants were included in this study, of which 33 achieved a live birth. Baseline characteristics are presented in Table 1. The mean age of the participants was 36 years (SD 4). Women who had a live birth had a mean age of 35 years (SD 5) compared to those who did not with a mean age of 37 years (SD 4) ($p = 0.089$). Body mass index (BMI) did not differ between the two groups, respectively 24 kg/m^2 (SD 3) and 24 kg/m^2 (SD 3) for the women who had a live birth and those who did not ($p = 0.604$). Smoking habits were alike amongst participants who achieved a live birth (12%) compared

Table 1 Baseline characteristics of the study population

	All **Mean (SD)** ***n*** **= 110**	**Live Birth: No** **Mean (SD)** ***n*** **= 77**	**Live Birth: Yes** **Mean (SD)** ***n*** **= 33**	***P*-value**
Age, (years)	36 (4)	37 (4)	35 (5)	0.089
BMI, (kg/m^2)	24 (3)	24(3)	24 (3)	0.604
Smoking, no. (%)	7 (6%)	3 (4%)	4 (12%)	0.240
ICSI treatment, no. (%)	59 (54%)	36 (47%)	23 (70%)	**0.045**
Male infertility, no. (%)	62 (56%)	39 (50%)	23 (70%)	0.102
Female infertility, no. (%)	89 (81%)	62(81%)	27 (82%)	1.000
Follicular phosphate (mmol/L)	1.18 (0.2)	1.18 (0.2)	1.18 (0.2)	0.915
Follicular calcium (mmol/L)	2.23 (0.2)	2.24 (0.2)	2.21 (0.2)	0.876
Follicular testosterone (nmol/L)	27.1 (21.7;32.2)	26.3 (21.2;33.8)	27.6 (23.5;31.6)	0.928
Serum phosphate (mmol/L)	1.06 (0.2)	1.04 (0.2)	1.10 (0.2)	0.335
Serum calcium (mmol/L)	2.36 (0.1)	2.36 (0.1)	2.36 (0.1)	0.832
Serum testosterone (nmol/L)	1.26 (0.93;1.91)	1.32 (0.87;1.92)	1.19 (0.94;1.53)	0.864

Data from either the women's first visit or the visit in which they achieved a pregnancy that led to a live birth. Data is presented as mean (SD) except testosterone which is presented as median (IQR). For serum, $n = 38$ for all, $n = 29$ for live birth: no, and $n = 9$ for live birth: yes

BMI Body mass index, *ICSI* Intracytoplasmic sperm injection, *IQR* Interquartile range

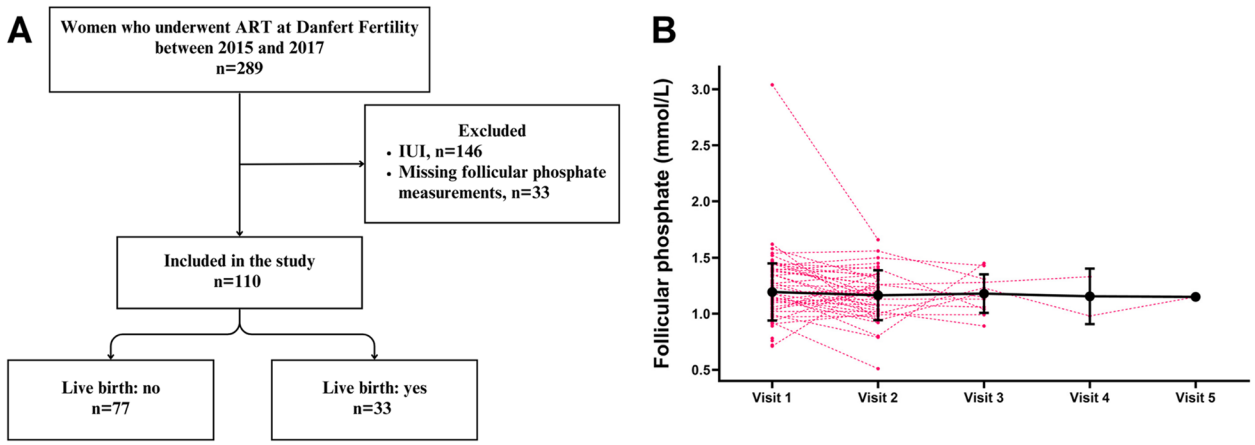

Fig. 1 Flowchart and follicular phosphate concentrations according to each visit. **A** Flowchart of participant selection for the study. ART: Assisted reproductive treatment, IUI: Intrauterine insemination **B** Each study participant's follicular phosphate concentration at each visit. The red dotted line represents each participant, while the thick black line represents the study participants' mean follicular phosphate concentration at each visit with standard deviation. Number of participants at visit no. 1: 110, visit no. 2: 34, visit no. 3: 12, visit no. 4: 2, visit no. 5: 1

to those who did not (4%) ($p = 0.240$). A significantly higher proportion of participants who achieved a live birth underwent ICSI treatment (70%) compared to those who did not (47%) ($p = 0.045$). There were no differences in the proportions of male and female infertility diagnoses between the two groups (male, $p = 0.102$ and female, $p = 1.000$). The mean follicular phosphate concentration was 1.17 mmol/L averaged for the first visit across participants. Women having multiple aspirations at different time points had a stable serum phosphate over time ($p = 0.468$), (Fig. 1b).

Table 2 Mean difference in follicular fluid and serum phosphate concentrations

	n	Mean difference	*P*-value	95% CI
All	38	0.10 mmol/L	**0.002**	0.043; 0.168
Live Birth: No	29	0.11 mmol/L	**0.030**	0.030; 0.188
Live Birth: Yes	9	0.096 mmol/L	0.058	−0.195; 0.004

Mean difference is calculated as the mean phosphate concentration difference between follicular fluid and serum. Numbers in this table represent only the 38 women who had corresponding serum samples

CI Confidence interval

Phosphate, calcium, and testosterone concentrations in serum and follicular fluid

The concentrations of phosphate, calcium, and testosterone in follicular fluid and serum were measured and are presented in Table 1. The mean follicular fluid concentrations of phosphate (1.18 vs. 1.18 mmol/L, $p = 0.915$), calcium (2.24 vs. 2.21 mmol/L, $p = 0.876$), and median testosterone concentrations (26.3 vs. 27.6 nmol/L, $p = 0.928$) were similar across groups. Similarly, in serum, mean phosphate (1.04 vs. 1.10 mmol/L, $p = 0.345$), calcium (2.36 mmol/L in both groups, $p = 0.932$), and median testosterone (1.32 vs. 1.19 nmol/L, $p = 0.864$) concentrations showed no significant differences. Differences between serum and follicular fluid phosphate are shown in Table 2. We found a statistically significant mean difference of 0.10 mmol/L, indicating that phosphate concentrations were significantly higher in follicular fluid compared to serum (1.16 mmol/L vs. 1.06 mmol/L, $p = 0.002$). When stratified by live birth outcome, the subgroup of 29 patients without a live birth also exhibited a statistically significant difference in phosphate concentrations, with a mean difference of 0.11 mmol/L ($p = 0.030$). In contrast, the subgroup of 9 patients with a live birth showed a smaller and not significant mean difference of 0.096 mmol/L ($p = 0.058$).

Correlation between serum and follicular fluid mineral concentrations

Correlation analyses were performed to assess the relationships between phosphate, calcium, and testosterone concentrations in serum and follicular fluid (Fig. 2). There was a significant positive correlation between serum and follicular fluid phosphate concentrations ($r = 0.43$, $p = 0.007$). A similar positive correlation was observed for calcium but did not reach significance ($r = 0.31$, $p = 0.060$). Moreover, no correlation between serum and follicular fluid testosterone concentrations ($r = 0.08$, $p = 0.650$) was found. There was a positive correlation between phosphate and calcium concentrations in follicular fluid ($r = 0.41$, $p < 0.001$) (Fig. 3). In contrast, the correlation between phosphate and calcium concentrations in serum was not statistically significant

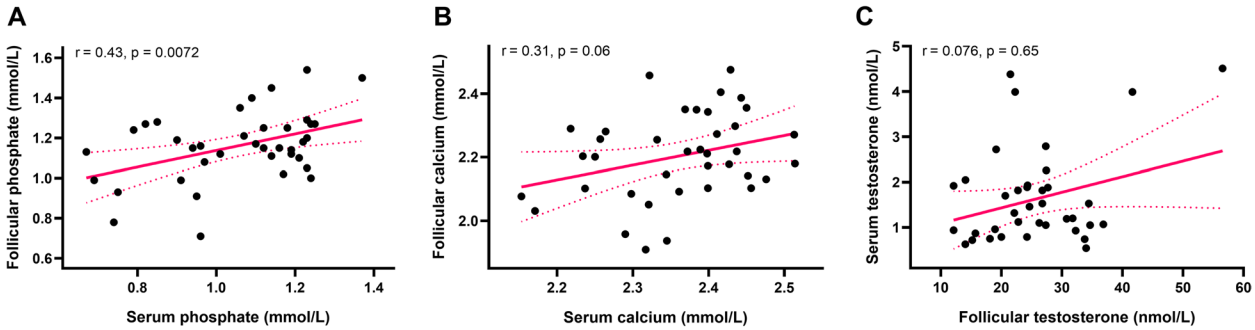

Fig. 2 Correlation of follicular and serum concentrations of phosphate, calcium, and testosterone. Correlation of serum and follicular fluid concentrations. Dotted lines represent 95% confidence intervals. **A** Correlation of serum and follicular fluid phosphate (Pearson) **B** Correlation of serum and follicular phosphate calcium (Pearson), **C** Correlation of follicular fluid and serum testosterone (Spearman)

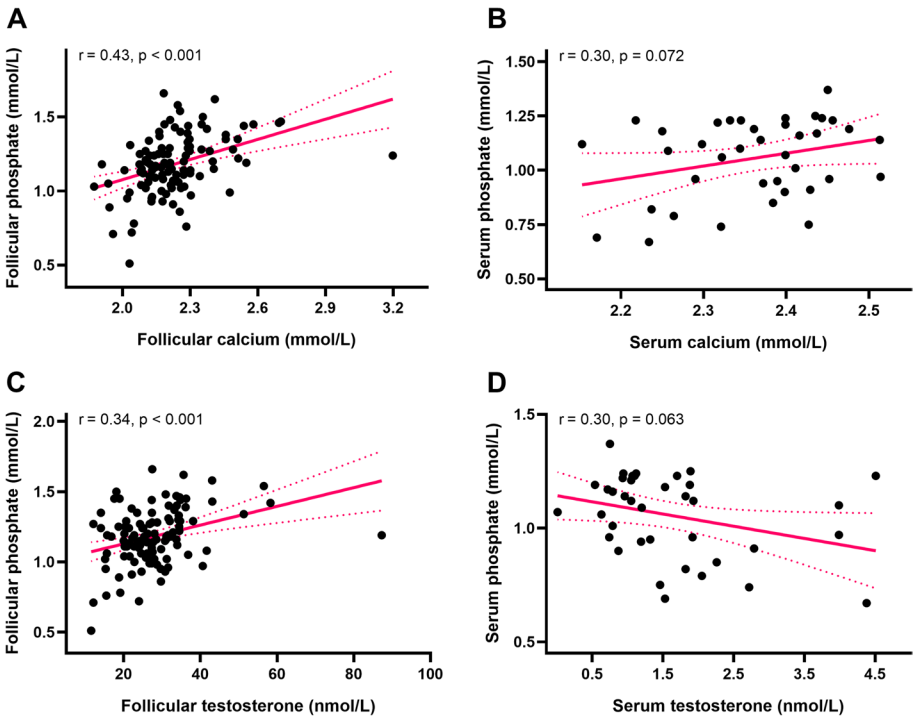

Fig. 3 Correlations of phosphate with calcium and testosterone in follicular fluid and serum. Correlation of serum and follicular fluid concentrations. **A** Correlation of phosphate and calcium in follicular fluid (Pearson) **B** Correlation of phosphate and calcium in serum (Pearson) **C** Correlation of phosphate and testosterone in follicular fluid (Spearman), **D** Correlation of phosphate and testosterone in serum (Spearman)

(r =0.29, p= 0.070). A significant positive correlation was also observed between phosphate and testosterone concentrations in follicular fluid (r =0.34, p< 0.001). In serum, there was a trend toward an inverse correlation between phosphate and testosterone concentrations, however, it was not statistically significant (r =−0.30, p= 0.060). Correlation analysis based on phosphate and baseline anthropometric data showed no correlation between phosphate and age (r =−0.09, p= 0.36) or BMI (r =−0.09, p= 0.35).

Tertile distribution in relation to pregnancy outcomes

We stratified the participants into tertiles based on follicular phosphate, calcium, and testosterone concentrations, in relation to live birth rate (Table 3). For phosphate, the highest live birth rate was in the middle tertile (34.2%) and the lowest in the first tertile (25.6%), though this was not statistically significant (p = 0.624). For calcium, the lowest tertile showed a higher live birth rate (40.5%) compared to the highest tertile (22.2%), but this trend was not significant (p = 0.207). Testosterone

Table 3 Follicular phosphate, calcium, and testosterone concentrations stratified according to tertiles

	Live birth: No	Live birth: Yes	*P*-value
Follicular phosphate			
Lowest tertile	74%	26%	0.624
Middle tertile	66%	34%	
Highest tertile	70%	30%	
Follicular calcium			
Lowest tertile	60%	40%	0.207
Middle tertile	73%	27%	
Highest tertile	78%	22%	
Follicular testosterone			
Lowest tertile	73%	27%	0.841
Middle tertile	67%	33%	
Highest tertile	70%	30%	

Live birth rates when data was stratified in tertiles. Differences between tertiles was tested using Chi^2-test

tertiles had similar live birth rates ranging from 27% to 29.7%, ($p = 0.841$). To investigate whether live birth rates were dependent on both calcium and phosphate concentrations in the follicular fluid participants were classified into two categories: those in the lowest calcium tertile and/or in the middle or high phosphate tertile and those in the middle or high calcium tertile and the lowest phosphate tertile (Table 4). In the combined group (lowest calcium tertile or middle/high phosphate tertile), 30 out of 91 participants (33%) achieved a live birth, compared to 3 out of 19 participants (16%) in the rest of the cohort. Despite this numerical difference, the results did not reach statistical significance ($p = 0.111$).

Discussion

This study shows that follicular phosphate concentrations are positively correlated with serum phosphate, which suggests that follicular phosphate may be susceptible to dietary changes or therapies that regulate phosphate absorption, renal excretion, or mobilization from storage such as the skeleton. Despite the correlation with serum phosphate concentration, follicular phosphate concentration was significantly higher than serum concentration in women who did not achieve a live birth, while this was not the case for women who achieved a live birth. The lack of significance in the live birth group may be a chance finding due to the small sample size and should only be considered exploratory and warrants further investigation.

Although the absolute difference in follicular phosphate concentrations between serum and follicular fluid is modest (0.11 mmol/L), this finding may still hold biological significance. Systemic phosphate regulation is tightly maintained by hormones such as parathyroid hormone, FGF23, and active vitamin D [11], and even minor deviations can influence physiological processes. This difference may serve as a marker for increased cell turnover, as elevated follicular phosphate concentrations may be a result of the release of intracellular phosphate into the follicular fluid. Intracellular inorganic phosphate concentrations do not diverge significantly from those in serum, with values generally ranging from 0.7 to over 2.0 mmol/L [19]. However, a large fraction of phosphate intracellularly is available as organic phosphate that also can be released and may contribute to the higher phosphate content during cell lysis. To the best of our knowledge, follicular phosphate concentrations have not previously been reported in humans, although studies in animal models have shown comparable phosphate concentrations in serum and follicular fluid [20].

The precise mechanisms governing the transport of minerals such as phosphate across granulosa cells into the follicular fluid are not yet fully understood. A clinical study with tritium-labeled injections revealed the presence of tritium in follicular fluid an hour post injection, suggesting that follicular fluid composition is influenced by serum composition [21]. Nevertheless, our results could imply an active transport process of phosphate into the follicle with a tightly regulated phosphate environment in the follicular fluid. This aligns with the current understanding that follicular fluid is a complex mixture of serum-derived components and locally synthesized factors within granulosa cells [22]. If phosphate is actively transported into the follicle, low serum phosphate concentrations could lead to

Table 4 Live birth rates

	Live birth: No	Live birth: Yes	Total	*P*-value
Follicular calcium in the lowest tertile and/or follicular phosphate in the middle or highest tertile	61 (79%)	30 (91%)	91	0.111
Follicular calcium in the middle or highest tertile and follicular phosphate in lowest tertile	16 (21%)	3 (9%)	19	
Total	77	33		

Birth rates in the group who had either a follicular fluid calcium concentration in the lowest tertile and/or a follicular fluid phosphate concentration in the middle or highest tertile, and the group who had calcium concentration in the middle or highest tertile and a phosphate concentration in the lowest tertile, respectively. Differences between groups was tested using Chi^2-test

correspondingly low phosphate concentrations in follicular fluid due to limited availability (Fig. 2A). In the absence of direct evidence from our study, these interpretations remain hypothetical, underscoring the need for future mechanistic investigations to elucidate the pathways of phosphate regulation in the follicular environment. The moderate correlation between follicular and serum phosphate ($r = 0.43$, $p = 0.007$) suggests that while systemic influences are evident, local regulatory mechanisms within the follicular microenvironment may also be important. Although analogous examples in the female reproductive tract are limited, observations from other systems—such as the precise control seen in renal and skeletal mineral homeostasis—underscore that small differences in mineral concentrations can have significant biological impacts. Further research is warranted to delineate the relative contributions of systemic versus local regulation on follicular phosphate levels.

In this study, we identified a positive correlation between phosphate and testosterone in follicular fluid, contrasting with a trend toward a negative correlation in serum. The correlation between phosphate and testosterone in follicular fluid might be influenced by the stimulation protocols applied during ARTs. Some women are treated only with FSH while others are treated with both luteinizing hormone/human chorionic gonadotropin (hCG) combined with FSH, which have different stimulatory effects on the follicle. While the serum correlation did not reach statistical significance – likely due to the small sample size – the lack of correlation between serum and follicular fluid testosterone concentrations has been observed previously by a study on women in natural menstrual cycles which also found no association between serum and follicular fluid testosterone [23]. Studies have previously demonstrated age-related declines in serum testosterone and lower testosterone concentrations associated with a reduced number of secondary follicles [4, 24, 25]. We did not find this correlation, possibly, as our cohort had a quite narrow age range. Furthermore, the observed positive correlation between follicular phosphate and testosterone may be mechanistically linked to the role of phosphate in cellular energy metabolism. Phosphate is essential for ATP production, a key driver of the energy-demanding process of steroidogenesis in the ovarian follicle. Enhanced ATP production could facilitate increased steroidogenic activity in theca cells, thereby elevating local testosterone synthesis. Nonetheless, as no previous studies have directly investigated this relationship in follicular fluid, these interpretations remain speculative. Future mechanistic studies are needed to elucidate whether phosphate directly influences testosterone production through metabolic pathways in the follicular microenvironment.

The trend towards a possibly favorable high-concentration phosphate and low-concentration calcium in the follicle seems to be the opposite in the male reproductive tract. In male reproductive health, the role of phosphate is not completely understood although recent data suggests a role in both testicular and epididymal function [26, 27]. Remarkably, seminal fluid contains phosphate at concentrations several fold higher than in serum [14]. Semen quality has been shown to be negatively influenced by reduced seminal fluid concentration of both calcium and phosphate [28, 29], while the presence of phosphate and calcium during in vitro fertilization may increase the competence and efficiency of the sperm [30]. This divergence may be attributed to fundamental differences in the physiological roles and regulatory mechanisms governing mineral homeostasis in the female and male reproductive systems, which are likely tailored to support the distinct requirements of oogenesis and spermatogenesis, respectively. Therefore, the role of the minerals in the reproductive tract is complex, and whether phosphate can play a role in oocyte quality in the female reproductive tract needs to be explored.

Our study's primary strength is the novelty of measuring phosphate in follicular fluid together with other minerals and hormones. However, some limitations exist for instance the pooling of follicular fluid into a single composite sample per cycle per participant; thus, this may mask important variations between individual follicles. Future studies could benefit from analyzing individual follicular fluid samples to identify biomarkers or mechanisms linked to oocyte quality, which may be obscured in pooled analyses. This could enhance personalized fertility treatments and improve ART outcomes. Additionally, variability in the timing of serum sample collection presents a limitation; samples were obtained either on the day of oocyte aspiration or up to two weeks earlier. This variability may explain the difference in follicular fluid and serum concentrations of measured minerals. Despite these constraints, the use of mixed linear regression models demonstrated significant stability in mineral measurements over time for the same individual, and strong correlations between compartments support the robustness of our findings. Clinical data for participants were extracted from routine patient records, reflecting the standard workflow in a private fertility clinic. Stratifying participants by conditions such as polycystic ovarian syndrome, rather than focusing solely on ovulatory status, could provide greater clarity in regard to the regulatory mechanisms of minerals. This study is limited by the relatively small number of live births and the subset of participants with paired serum samples, both of which

may reduce statistical power and introduce potential selection bias. Consequently, some findings should be interpreted with caution, as Type II errors cannot be ruled out. Future studies with larger, well-powered cohorts and more comprehensive sampling are needed to validate these observations. Due to the exploratory nature of this study, no formal corrections for multiple testing were applied. Therefore, the results should be interpreted with caution, considering the increased risk of type I errors. Future studies may benefit from a design with sufficient power to demonstrate clinical significance, ideally through a randomized controlled trial (RCT). Potential confounding factors, such as dietary phosphate intake, vitamin D status, FGF23, parathyroid hormone levels, or ICSI treatment were not accounted for in our study. These variables could significantly impact both systemic and follicular phosphate levels and should be considered in future research to determine the clinical relevance of our findings more accurately.

In conclusion, our findings suggest that follicular phosphate levels are influenced by systemic phosphate homeostasis, but their role in ART outcomes remains unclear. Future studies with larger sample sizes and mechanistic investigations are required to determine potential clinical relevance.

Abbreviations

IVF	In vitro fertilization
ICSI	Intracytoplasmic sperm injection
ART	Assisted reproductive treatment
FSH	Follicle-stimulating hormone
PTH	Parathyroid hormone
FGF23	Fibroblast growth factor 23
CV	Coefficient variation
SD	Standard deviation
IUI	Intrauterine insemination
hCG	Human chorionic gonadotropin

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Authors' contributions
S.M.W. reviewed the literature, organized the writing, and wrote the initial draft. S.M.W., M.J.J., S.K.Y. and M.B.J. designed the study and directed the analyses, which were carried out by S.M.W. In line with the mentioned authors, I.K., U.B.L and H.K. participated in the discussion and interpretation of the results, critically revised the manuscript for intellectual content, and approved the final version. S.M.W. is the guarantor of this work and, as such, had full access to all data in the study and takes responsibility for data integrity. Authors M.B.J. and S.K.Y. contributed equally to this study.

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Data availability
The datasets generated and analyzed during this study are not publicly available to protect patient confidentiality but can be obtained from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
Written consent was collected from each patient after providing full information about the study's purpose and procedures. The study was approved by the Regional Ethical Committee (H-15000931).

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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