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CORRELATION BETWEEN SERUM PROLIDASE ACTIVITY AND SELECTED VITAMIN DEFICIENCIES IN WOMEN WITH PRIMARY AND SECONDARY INFERTILITY

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ABSTRACT

Overview: Female infertility is a complex condition caused by many factors including oxidative stress, depleted antioxidants, unbalanced micronutrients, changes to the extracellular matrix and inflammation in the reproductive tract that can disrupt normal functioning of both the ovaries and uterus.

Aims: To determine whether there were any associations between levels of prolidase activity and certain nutrients (vitamins A, C, and E; copper; selenium; ceruloplasmin [an antioxidant copper-carrying protein]) in serum among fertile and infertile (primary and secondary) women as well as determine if there are any correlations between both groups with regard to each variable being analysed.

Methods: This observational case-control biochemical study was conducted among women aged 18-35 years attending medical clinics in Samarra city during September 2021-April 2022. The study groups were fertile women as a control group, primary infertility women, and women with secondary infertility. Serum prolidase activity, vitamin D, vitamin A, vitamin C, vitamin E, total antioxidant capacity (TAC), reduced glutathione (GSH), malondialdehyde (MDA), ceruloplasmin (Cp), and selenium (Se) were assessed. Group comparisons and Pearson correlation analyses were reported according to the supplied dataset. Statistical significance was considered at $p < 0.05$ and high significance at $p < 0.01$.

Results: In the source file, infertile groups showed higher serum prolidase activity and MDA, and lower vitamin D, TAC, GSH, ceruloplasmin, selenium, vitamin A, and vitamin C compared with fertile women. In primary infertility, TAC showed a significant negative correlation with MDA ($r = -0.388$, $p = 0.034$), while serum prolidase activity was not significantly associated with the other tested biochemical parameters. In secondary infertility, serum prolidase activity showed a significant positive correlation with vitamin A ($r = 0.459$, $p = 0.011$). Vitamin D was negatively correlated with age ($r = -0.438$, $p = 0.015$), whereas vitamin E was positively correlated with age ($r = 0.399$, $p = 0.029$).

Conclusion: The findings suggest that women with infertility in this dataset had evidence of oxidative-antioxidant imbalance and selected vitamin alterations. Prolidase activity showed a different biochemical correlation pattern in secondary infertility compared with primary infertility. The study demonstrates associations only and does not establish causality.

Keywords: Infertility; Primary Infertility; Secondary Infertility; Vitamin D

1 INTRODUCTION

Infertility is a disease of the male or female reproductive system defined by failure to achieve pregnancy after 12 months or more of regular unprotected sexual intercourse [1]. Contemporary reproductive medicine guidance also recognizes infertility as the inability to achieve a successful pregnancy based on reproductive history, age, physical findings, diagnostic testing, or the need for medical intervention [2]. The clinical significance of this condition includes its effects on reproductive autonomy, psychosocial well-being, family planning and access to specialized care. Infertility is evaluated through the integration of several factors relating to ovulatory function, the uterine and the tube, endocrinology, The male partner and unexplained factors [3,4].

When referring to female infertility, it is common to classify it as either primary or secondary. Primary infertility refers to being unable to conceive a first pregnancy, while secondary infertility refers to having difficulty conceiving after having previously conceived. Although these two categories may overlap at times when a person is trying to conceive, they can also reflect more than one possible biological issue, including ovulatory dysfunction, tubal disease, endometriosis, uterine abnormalities, past pelvic infections, metabolic disorders, nutrition issues, or other undefined causes. [3,4].

Oxidative stress has an important role within female reproductive physiology and pathology. Reactive oxygen species are involved in several aspects of reproductive function including follicular development, oocyte maturation, ovulation, luteal function, fertilization, embryonic development, and implantation, when they are within physiological limits, but there is an excess production of free radicals and/or a lack of antioxidant mechanisms, which will lead to impairment of reproductive function [5-8].

Oxidative stress has been associated with decreased oocyte quality, dysfunctional granulosa-cells, mitochondrial damage, abnormal steroid hormone production, defective endometrial receptivity, failure of implantation, and inflammatory reproductive conditions [5-8]. Prolidase is an exopeptidase located in the cytosol, and it hydrolyses imidodipeptides with a proline or hydroxyproline residue at the C-terminus. Prolidase participates in the turnover of collagen, remodeling of the extracellular matrix, recycling of proline, repair of tissues, and remodeling due to inflammation [9,10]. Because ovarian and endometrial tissues undergo repeated cycles of extracellular matrix degradation and repair, altered prolidase activity may provide biochemical insight into reproductive tissue turnover. Previous studies have reported increased serum prolidase activity and oxidative stress in polycystic ovary syndrome and unexplained infertility, supporting the relevance of prolidase to reproductive biochemical imbalance [11,12].

Selected vitamins and trace elements may also influence reproductive physiology. Vitamin D has been associated with ovarian and endometrial function and with assisted reproductive treatment outcomes [13]. Vitamin A and retinoic acid signaling are required for epithelial integrity, folliculogenesis, oocyte maturation, and ovarian steroidogenic regulation [14,15]. Vitamin E is a lipid-soluble antioxidant with membrane-protective properties in reproductive tissues [16]. Glutathione is a major intracellular antioxidant, selenium supports selenoprotein-dependent antioxidant enzymes, MDA reflects lipid peroxidation, and ceruloplasmin participates in copper transport, ferroxidase activity, and oxidative-inflammatory balance [17-20].

The present study aimed to examine serum prolidase activity and selected vitamin deficiencies in women with primary and secondary infertility, and to evaluate their correlation with oxidative stress markers, antioxidant defense indices, selenium, ceruloplasmin, age, and BMI.

2 MATERIALS AND METHODS

2.1 Study design and setting

This observational case-control biochemical study was conducted among women attending medical clinics in Samarra city during the period from September 2021 to April 2022.

2.2 Study population

Participants were women aged 18-35 years. The study groups were fertile women serving as controls, women with primary infertility, and women with secondary infertility. The samples were collected from women attending medical clinics and that direct interviews/questionnaires were used to obtain participant information.

2.3 Blood sampling and Biochemical assessment

Venous blood samples were collected under clinical conditions, and serum was prepared for biochemical measurements.

The biochemical variables included serum prolidase activity, vitamin D, vitamin A, vitamin C, vitamin E, total antioxidant capacity, reduced glutathione, malondialdehyde, ceruloplasmin, and selenium. All biochemical variables were measured using ELISA kits (Sunlong, China) according to the manufacturer instructions. Age and BMI were also included.

2.4 Statistical analysis

Data were presented as mean $\pm$ standard deviation where available. Comparisons among fertile women, women with primary infertility, and women with secondary infertility were reported according to t test. Pearson correlation analysis was used to examine associations among variables in women with primary infertility and women with secondary infertility. A p-value < 0.05 was considered statistically significant, and $p < 0.01$ was considered highly significant.

3 RESULTS

3.1 Demographic characteristics

As shown in Table 1, the reported mean age and BMI were comparable among fertile women, women with primary infertility, and women with secondary infertility.

Table 1: Demographic characteristics of the studied groups

Parameter	Fertile women	Primary infertility	Secondary infertility	p-value
Age (years)	27.53 $\pm$ 3.77	26.64 $\pm$ 4.01	28.72 $\pm$ 4.47	>0.05
BMI (kg/m ²)	24.16 $\pm$ 2.37	24.03 $\pm$ 1.86	25.12 $\pm$ 2.59	>0.05

Values are presented as mean $\pm$ SD as reported in the source file. BMI = body mass index. *p < 0.05 ; **p < 0.01 .

3.2 Serum prolidase activity, vitamins, trace elements, and oxidative stress markers

Table 2: Serum prolidase activity, vitamins, trace elements, and oxidative stress markers among fertile, primary infertile, and secondary infertile women

Parameter	Fertile women	Primary infertility	Secondary infertility	p-value
Prolidase (U/L)	322.63 $\pm$ 124.95	537.51 $\pm$ 207.34	506.35 $\pm$ 198.26	<0.01**
Vitamin D (ng/mL)	25.78 $\pm$ 3.55	15.20 $\pm$ 3.71	16.92 $\pm$ 3.15	<0.01**
TAC (μ mol vitamin C Eq/L)	1.79 $\pm$ 0.49	0.71 $\pm$ 0.38	0.79 $\pm$ 0.38	<0.01**
GSH (μ mol/L)	2597.83 $\pm$ 512.56	964.30 $\pm$ 579.90	1322.92 $\pm$ 562.83	0.015*
MDA (μ mol/L)	0.47 $\pm$ 0.09	1.42 $\pm$ 0.59	1.38 $\pm$ 0.45	<0.01**
Ceruloplasmin (mg/dL)	26.86 $\pm$ 4.56	19.03 $\pm$ 7.76	19.44 $\pm$ 5.03	<0.01**
Selenium (μ g/L)	136.61 $\pm$ 34.52	108.93 $\pm$ 30.42	122.94 $\pm$ 29.77	<0.01**
Vitamin A (μ mol/L)	1.40 $\pm$ 0.25	0.81 $\pm$ 0.27	0.73 $\pm$ 0.30	<0.01**
Vitamin C (μ g/mL)	14.39 $\pm$ 4.58	6.51 $\pm$ 3.56	7.12 $\pm$ 5.18	<0.01**
Vitamin E (μ g/mL)	11.59 $\pm$ 2.76	11.01 $\pm$ 3.22	11.06 $\pm$ 3.22	>0.05

Values are presented as mean $\pm$ SD exactly as reported in the source file. TAC = total antioxidant capacity; GSH = reduced glutathione; MDA = malondialdehyde; Cp = ceruloplasmin; Se = selenium. *p < 0.05 ; **p < 0.01 .

The comparison of biochemical parameters among the studied groups is presented in Table 2. Serum prolidase activity was higher in women with primary infertility and secondary infertility than in fertile women. Vitamin D, TAC, GSH,

ceruloplasmin, selenium, vitamin A, and vitamin C were lower in the infertile groups than in fertile women, while MDA was higher in the infertile groups. Vitamin E did not show a significant difference according to the p-value.

3.3 Correlation analysis in primary infertility

Pearson correlation analysis in women with primary infertility is shown in Table 3. In women with primary infertility, TAC showed a significant negative correlation with MDA ($r = -0.388$, $p = 0.034$). No significant correlation was observed between serum prolidase activity and the other tested biochemical parameters in women with primary infertility. The significant inverse association between TAC and MDA is illustrated in Figure 1.

Table 3: Pearson correlation matrix in women with primary infertility

Parameter	Vitamin D	Vitamin A	Vitamin C	Vitamin E	TAC	GSH	MDA	Cp	Se
Prolidase	0.021	-0.264	0.111	-0.188	-0.156	0.054	-0.155	-0.072	0.121
Vitamin D	—	-0.028	-0.127	-0.076	0.166	0.008	0.237	0.288	0.105
Vitamin A	-0.029	—	0.077	0.048	-0.135	0.034	0.135	-0.262	-0.076
Vitamin C	-0.127	0.077	—	-0.047	0.251	0.101	-0.260	0.058	-0.135
Vitamin E	-0.075	0.048	-0.048	—	0.321	0.034	-0.234	0.006	0.209
TAC	-0.166	-0.137	0.250	0.321	—	-0.029	-0.388*	0.097	-0.061
GSH	0.008	0.034	0.103	0.034	-0.028	—	-0.234	-0.016	0.204
MDA	0.238	0.135	-0.262	-0.235	-0.388*	-0.237	—	-0.078	0.094
Cp	0.288	-0.263	0.058	0.006	0.097	-0.016	-0.078	—	-0.267
Se	0.103	-0.076	-0.137	0.208	-0.060	0.202	0.094	-0.267	—
Age	-0.219	-0.128	-0.047	-0.256	-0.079	-0.104	0.253	-0.031	0.051
BMI	-0.135	0.018	-0.080	-0.120	-0.178	0.357	0.059	-0.293	0.304

Values represent Pearson correlation coefficients exactly as presented in the source file. BMI = body mass index; TAC = total antioxidant capacity; GSH = reduced glutathione; MDA = malondialdehyde; Cp = ceruloplasmin; Se = selenium. * $p < 0.05$. Significant correlations.

3.4 Correlation analysis in secondary infertility

Pearson correlation analysis in women with secondary infertility is shown in Table 4. Serum prolidase activity showed a significant positive correlation with vitamin A ($r = 0.459$, $p = 0.013$). Vitamin D showed a significant negative correlation with age ($r = -0.438$, $p = 0.015$), while vitamin E showed a significant positive correlation with age ($r = 0.399$, $p = 0.029$). These associations are illustrated in Figures 2-4.

Table 4: Pearson correlation matrix in women with secondary infertility

Parameter	Vitamin D	Vitamin A	Vitamin C	Vitamin E	TAC	GSH	MDA	Cp	Se
Prolidase	-0.087	0.459*	-0.328	-0.017	-0.184	0.162	-0.173	0.168	0.118
Vitamin D	—	0.045	-0.075	-0.064	0.146	-0.201	0.002	-0.188	-0.094
Vitamin A	0.045	—	-0.291	-0.321	-0.234	-0.031	-0.244	0.204	0.174
Vitamin C	-0.076	-0.292	—	0.271	0.077	-0.178	0.077	-0.231	-0.158
Vitamin E	-0.065	-0.321	0.272	—	-0.276	-0.031	0.321	-0.091	-0.027
TAC	0.146	-0.233	0.077	-0.278	—	0.127	-0.134	0.043	-0.304
GSH	-0.201	-0.032	-0.178	-0.030	0.127	—	-0.245	0.125	-0.093
MDA	-0.002	-0.244	0.077	0.323	-0.134	-0.245	—	0.026	0.315
Cp	-0.188	0.204	-0.231	-0.091	0.041	0.125	0.025	—	0.167
Se	-0.095	0.171	-0.158	-0.027	-0.302	-0.093	0.313	0.167	—
Age	-0.438*	-0.039	-0.021	0.399*	-0.211	0.079	-0.012	0.311	0.205
BMI	-0.084	0.223	-0.076	0.031	-0.075	0.015	-0.255	0.152	-0.167

Values represent Pearson correlation coefficients exactly as presented in the source file. BMI = body mass index; TAC = total antioxidant capacity; GSH = reduced glutathione; MDA = malondialdehyde; Cp = ceruloplasmin; Se = selenium. * $p < 0.05$. Significant correlations.

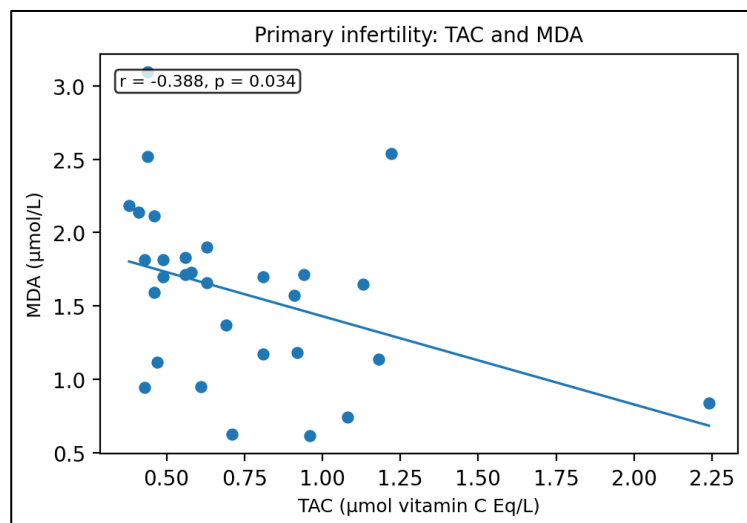


Figure 1: Correlation between TAC and MDA in primary infertile women.

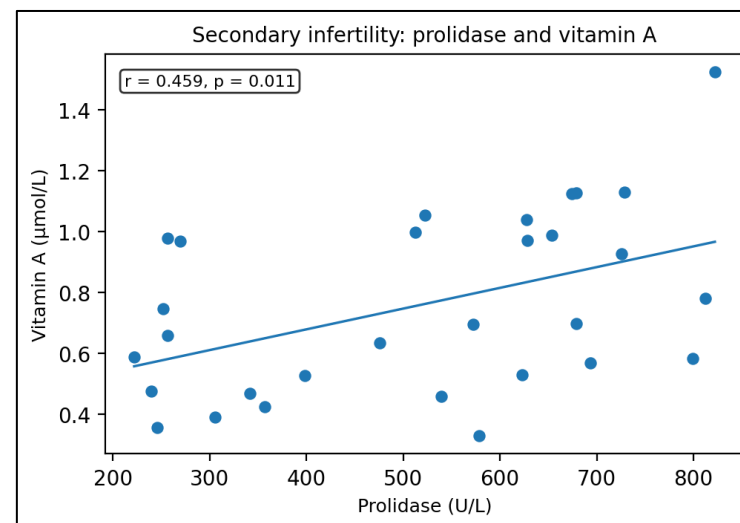


Figure 2: Correlation between serum prolidase activity and vitamin A in secondary infertile women.

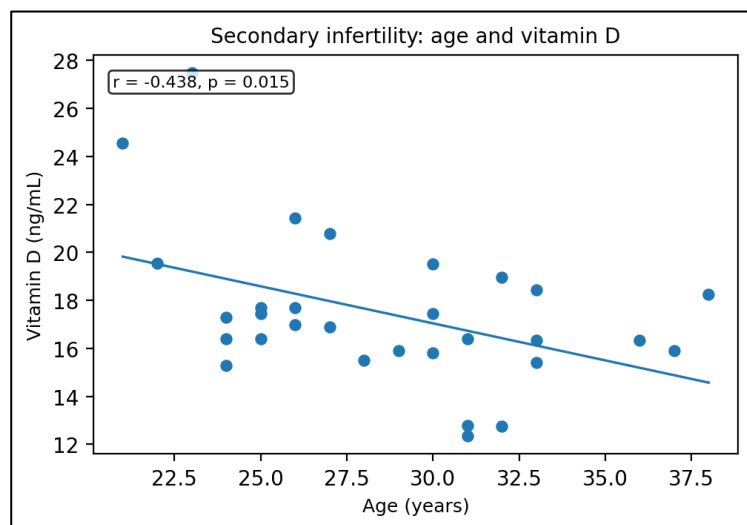


Figure 3: Correlation between age and vitamin D in secondary infertile women.

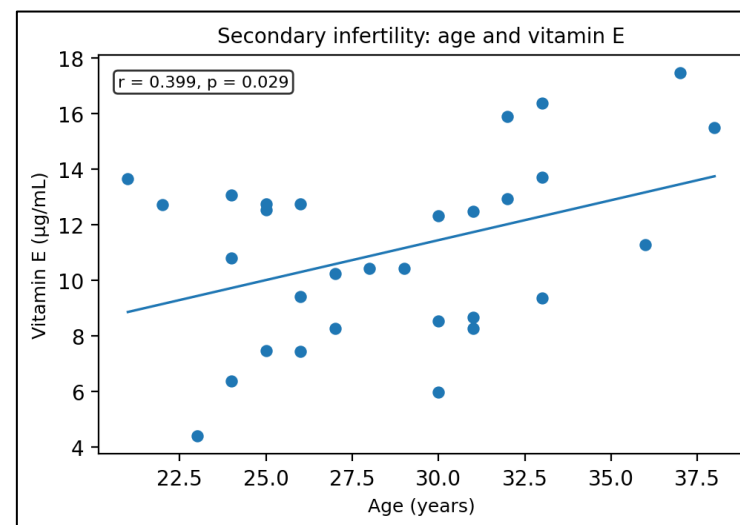


Figure 4: Correlation between age and vitamin E in secondary infertile women.

4 DISCUSSION

Women suffering from primary and secondary infertility were shown to have changes/ altered biochemical profiles with respect to serum Prolidase activity, selected vitamins, antioxidant defense markers, lipid peroxidation products, selenium, and ceruloplasmin. Significant correlation findings included a strong inverse relationship between Total Antioxidant Capacity (TAC) and Malondialdehyde (MDA) values in women experiencing primary infertility, no statistically significant relationship between Prolidase activity and the aforementioned biochemical variables for primary infertility group; versus several significant correlations involving Prolidase activity with Vitamin A and age with Vitamin D, and age with Vitamin E among women experiencing secondary infertility. The results of lower TAC and higher MDA levels align with evidence indicating oxidative stress plays a role in reproductive problems for females. Reactive oxygen species are needed for normal ovarian and endometrial functions; however, excess oxidative damage can and will damage egg quality, granulosa cell function, steroid hormone production, ability to accept and implant an embryo, and proper balance of reproductive inflammatory response[5-8]. Thus, the statistically significant negative correlation between TAC and MDA in the primary infertility group provides evidence that having a lower overall antioxidant capacity was associated with higher levels of lipid peroxidation.

MDA is the biomarker for lipid peroxidation and oxidative damage to fatty acids[19]. While TAC reflects the total integrated antioxidant capacity of circulating antioxidant systems while glutathione reflects the central thiol antioxidant and redox buffer that is involved with detoxification of peroxides and cellular protection[17]. Therefore, the coexistence of higher MDA with lower TAC and GSH suggests an oxidative-antioxidant imbalance in infertile women, although the present design cannot determine whether this imbalance preceded or followed infertility-related pathology.

Serum prolidase activity was higher in the infertile groups compared with fertile women in the source file. Prolidase is involved in collagen recycling and extracellular matrix remodeling, and fluctuations in its activity may indicate altered collagen metabolism, tissue turnover, or inflammatory remodeling [9,10]. Reproductive tissues are particularly dependent on controlled extracellular matrix remodeling during follicular rupture, corpus luteum formation, cyclic endometrial regeneration, implantation, and repair. Previous studies have also reported increased prolidase activity in polycystic ovary syndrome and unexplained infertility, suggesting a possible link between prolidase, oxidative stress, and reproductive tissue remodeling [11,12].

In primary infertility, prolidase activity was not significantly correlated with vitamin D, TAC, GSH, MDA, ceruloplasmin, selenium, vitamin A, vitamin C, or vitamin E. This absence of significant linear associations may indicate heterogeneity in the biochemical mechanisms underlying primary infertility. Primary infertility can arise from multiple etiologies, including ovulatory dysfunction, tubal disease, endometriosis, uterine factors, male factors, and unexplained causes [3,4]. Thus, elevated prolidase at the group level may not necessarily correspond to a single circulating vitamin or oxidative marker within a heterogeneous clinical subgroup.

In cases of secondary infertility, prolidase activity exhibited a notable positive correlation with vitamin A levels. This correlation must be interpreted with caution and not regarded as evidence of causation. Vitamin A and retinoic acid signaling are crucial for maintaining epithelial integrity, ovarian function, folliculogenesis, oocyte maturation, and steroidogenic regulation [14,15]. A positive correlation between prolidase and vitamin A may indicate interconnected roles in tissue remodeling, epithelial repair, extracellular matrix turnover, or the nutritional-inflammatory status in secondary infertility. Nonetheless, in the absence of dietary evaluation, inflammatory cytokines, reproductive hormones, and longitudinal measurements, the biological direction of this association remains ambiguous.

Women with secondary infertility had lower levels of vitamin D, and there was a strong negative link between vitamin D and age. Vitamin D is associated with ovarian and endometrial physiology and reproductive outcomes, especially in assisted reproductive contexts [13]. The age-related decrease in vitamin D in cases of secondary infertility may indicate variations in sunlight exposure, dietary consumption, adiposity, reproductive history, metabolic condition, or unquantified endocrine influences, rather than age alone. Due to the restricted age range of 18-35 years, this finding necessitates careful interpretation.

There was not a big difference in vitamin E levels between the groups, but there was a strong positive link between vitamin E levels and age in cases of secondary infertility. Vitamin E is an antioxidant that dissolves in fats and can protect cell membranes from lipid peroxidation [16]. The positive correlation with age may indicate dietary variability, supplement utilization, lipid transport disparities, or a coincidental association within a restricted sample. This finding should not be overinterpreted in the absence of supplement history and nutritional assessment.

The results showed that selenium, vitamin C, and ceruloplasmin levels were lower in women who were not able to get pregnant than in women who were able to get pregnant. Selenium is necessary for selenoproteins that protect against oxidative stress and are important for reproductive health [18]. Vitamin C helps protect against oxidative stress in water

and helps break down collagen, and ceruloplasmin may help keep the balance between oxidative and inflammatory processes by acting as a ferroxidase and moving copper around [20,21]. These changes further support the idea that there is a problem with the oxidative-antioxidant network, but they do not prove that a lack of nutrients caused infertility.

The study indicates that primary and secondary infertility exhibit overlapping yet distinct biochemical patterns. Primary infertility was primarily defined by the TAC-MDA relationship, while secondary infertility exhibited a unique prolidase-vitamin A association, along with age-related correlations with vitamin D and vitamin E. These results could be due to differences in reproductive history, tissue remodeling that happened during a previous pregnancy, exposure to inflammation, eating habits, or the cause of infertility. The results should be interpreted as associations derived from a case-control biochemical dataset.

5 CONCLUSION

The results indicated that women experiencing primary and secondary infertility exhibited biochemical markers of oxidative-antioxidant imbalance and specific vitamin alterations. In primary infertility, there was a strong negative correlation between TAC and MDA, but serum prolidase activity was not significantly linked to the other biochemical parameters that were tested. In secondary infertility, serum prolidase activity exhibited a significant positive correlation with vitamin A, a negative correlation with vitamin D and a positive correlation with vitamin E in relation to age. These results indicate that prolidase activity may exhibit a distinct biochemical relationship in secondary infertility relative to primary infertility.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

Disclosure of conflict of interest

The authors declare no conflicts of interest regarding this manuscript.

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