

## Special Article – Male Fertility

# Association of Cortisol and Vitamin D in Seminal Plasma with Sperm Quality

Pugliese MN<sup>1</sup>, Gonzalez D<sup>2</sup>, Jamardo JJ<sup>2</sup>, Ariagno JI<sup>1</sup>, Repetto H<sup>1</sup>, Jacobsen D<sup>2</sup>, Repetto EM<sup>2</sup>, Berg G<sup>2</sup>, Fabre B<sup>2#</sup> and Mendeluk GR<sup>1\*</sup>

<sup>1</sup>Laboratory of Male Fertility, Hospital de Clínicas "José de San Martín", Faculty of Pharmacy and Biochemistry, University of Buenos Aires, Argentina

<sup>2</sup>Clinical Biochemistry Department, INFIBIOC, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Cordoba 2351 (1120), Argentina

<sup>#</sup>Equally contributed

\*Corresponding author: Mendeluk Gabriela Ruth, Av. Cordoba 2351 (1120), Argentina

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## Abstract

**Objective:** To evaluate the relationship between Vitamin D (VD) and Cortisol (C) in seminal plasma (SP) and sperm quality.

**Design:** Ninety-one sperm samples from patients consulting for infertility analyzed according WHO and Computer Aided System. Normozoospermics (N, n=43); Pathological (P, n=48).

**Methods:** VD and C were measured by chemiluminescent methods. C was determined in saliva, serum, and SP while VD in the two latter ones in ten patients.

**Results:** C was  $1.48 \pm 0.56$  and  $1.85 \pm 0.79$  ug/dl while Vit D was  $27.5 \pm 10.3$  and  $28.52 \pm 7.74$  ng/ml in N and P seminal plasma (mean $\pm$ SD;  $p=0.020$  and NS respectively). A negative correlation was found between C and Progressive Motility (%) ( $r=-0.241$ ,  $p=0.03$ ) and sperm count ( $r=-0.395$ ,  $p<0.001$ ), being positive for lateral amplitude head displacement ( $r=0.258$ ,  $p=0.022$ ). VD did not correlate with any of the studied parameters. In the pathological samples VD in SP, correlated with total motility ( $r=0.350$ ,  $p=0.025$ ). C in SP represents approximately 10% of the serum concentration, with a higher concentration of VD in SP than in serum. A significant correlation was found between serum C and seminal plasma and saliva C ( $r=0.90$ ,  $p<0.001$  and  $r=0.81$ ,  $p=0.04$ , respectively). C in SP correlated with C in saliva ( $r=0.934$ ,  $p=0.001$ ). There was no correlation of the VD in serum and SP.

**Conclusions:** Cortisol would inversely affect sperm count and motility, imprinting a particular kinetics. In the pathological population, VD could have a non-genomic effect on sperm motility. Cortisol in SP as in saliva would be an ultrafiltrate of serum, while there would be local production of VD.

**Keywords:** Male Infertility; Psychosocial Stress; Sperm Kinetics; Seminal plasma; Cortisol; Vitamin D

## Introduction

Semen analysis is a testis, hormone status and male genital tract functional test. Data on sperm count, motility and morphology allow to characterize an infertile patient, as oligospermic, asthenozoospermic, teratozoospermic if deficiency of the respective parameters are observed. Combination of these features are frequently reported by the Andrology Laboratory. Normal spermatogenesis results in normal spermograms under hormonal hypothalamus-pituitary-gonadal axis, adequate regulation and non genital tract obstructions. Fertility achievement depends on the fertility potential of both couple partners, being in man reflected in his spermogram, which is still the main tool to board reproductive disorders in men [1-3].

Infertility is defined as the failure of conception for at least 12 months of unprotected intercourse [4]. It affects 15% of all couples trying to conceive. Male factor is the sole or contributing cause in roughly one-half of these cases [5]. Spermatogenesis, spermiogenesis and sperm capacitation are the three main axis while thinking in male fertility. Nearly 75% of its etiology may be attributed to testis disarrangements, probably because the natural physiology is yet not

completely understood.

Cortisol is the chemical expression of psychosocial stress [6]. Several researches have shown that individuals with fertility problems experience psychosocial problems [7]. The question of whether stress contributes to conception delay is a controversial issue that has received much attention in recent years, in part owing to the fact that despite advances in medicine some cases of infertility remain unexplained [8-10]. What is still unclear is the role that stress, defined as a physiological or psychological response to a positive or negative external stimulus, may play in reproductive function, in part due to an inability to separate cause and effect. Physiological compensatory linkages have been elucidated between the hypothalamic pituitary adrenal (HPA) axis and the hypothalamic pituitary gonadal axis [11].

Epidemiological studies of infertility in the developed world indicate that semen deficiencies in the male partner are the commonest diagnostic abnormality, with "asthenozoospermia" (percentage of progressive motile spermatozoa below the lower reference limit of 32%), a major cause probably implying different etiologies [12-14]. We have recently published a case report about a paradigmatic stressed patient in who a particular type of sperm

deficient movement, asthenozoospermia, was depicted, characterized by high energy but low progressiveness [15].

Vitamin D is a key element, regulating calcium homeostasis. More beyond its original role in bone metabolism it has been related to immune system, acting as immunosuppressor and antitumoral. Vitamin D receptors and 25-alpha hydroxylase and 1-alpha hydroxylase have also been detected in testis either in gonadal cells and Leydig ones, epididymis, prostate and mature sperm, having a neck localization [16-17]. This arguments support the hypothesis of its role in reproduction [16].

Both hormones are in close relation to life style and environmental factors. It has been described a negative correlation between cortisol and some chronic diseases like obesity, cardiovascular disease and cancer [18]. On the other hand Vitamin D has been related to immune system acting as immunosuppressor and antitumoral [19].

Our aim is to study possible relationship between Vitamin D and cortisol in seminal plasma and its impact on sperm quality.

## Materials and Methods

### Design

Cross sectional study among patients randomly selected.

### Subjects and methods

Ninety-one sperm samples from patients consulting for fertility were analyzed according to WHO standardization and by the employment of a Computer Aided System, thus being classified as normozoospermics (N, n=43) and not normozoospermics (P, n=48). Vitamin D and Cortisol were measured by chemiluminescent methods (Immulite 2000, Siemens, LA, USA and Advia Centaur XP, respectively). In ten men, cortisol was determined in saliva, serum, and seminal plasma and Vitamin D in the two latter ones.

### Sperm assays

Conventional sperm assay was performed according WHO criteria- 2010 [12]. A Sperm Class Analyzer CASA system (SCA Microptic SL Barcelona, Spain) was employed to assess kinetic parameters and sperm count. The basic components of the system are: a bright field microscope with phase contrast microscopy to visualize the sample (Nikon E- 200, Japan), a digital camera to capture images (Basler A312 Inc. Vision-Technology. Germany) and a computer with SCA® software installed. Samples were laid on a thermostatic plate at 37 °C. A minimum of 400 spermatozoons per sample were captured and the software analyzed 25 digitized images per second for each sperm. The kinetic assay was conducted in accordance with the standardization and validation of the instrument, using a Leja chamber 10 (10 microns in height, there was always a qualified operator who validated each analyzed image) [20]. Data from individual motile spermatozoa (Sp), defined by 8 kinematic parameters (curvilinear velocity [VCL], straight-line velocity [VSL], average path velocity [VAP], linearity [LIN], straightness [STR], mean amplitude of lateral head displacement [ALH], wobble [WOB] a measure of oscillation of the actual path about the average path and beat cross frequency [BCF]), were assessed and ratios were then calculated relating the different speeds (LIN = VSL/VCL; SRT = VSL/VAP, WOB=VAP/VCL).

### Statistics

Results are expressed as mean±standard deviation (SD) or median (range), according to the data distribution. Correlations between variables were calculated using Pearson (parametric distribution data) or Spearman test (non-parametric distribution data). P values of <0.05 were considered significant.

### Ethics

The present study was conducted according to the guidelines laid down in the Declaration of Helsinki and was approved by the Institutional Review Board of The Clinical Hospital "José de San Martín"; all the participants received information on the project and gave written informed consent to be included.

## Results

Cortisol in seminal plasmas from normospermic and pathological samples were 1.48±0.56 and 1.85±0.79 ug/dl and for Vitamin D 27.5±10.3 and 28.52±7.74 ng/ml and (mean±SD; p=0.020 and NS respectively) (Table 1). While analyzing the total population, a negative correlation was found between cortisol and Progressive Motility (%) (r=-0.241, p=0.03) and sperm count (r=-0.395, p<0.001), being positive for lateral amplitude head displacement (r=0.258, p=0.022). Vitamin D did not correlate with any of the studied parameters. In the pathological group, an inverse correlation was found between cortisol in seminal plasma and semen volume (r=-0.218, p=0.040) and positive one with the beat crossed frequency (r=0.318, p=0.04), while Vitamin D in seminal plasma correlated with Total Motility/ejaculate (r=0.350, p=0.025) (Table 2). Cortisol in seminal plasma represents approximately 10% of the serum concentration, with a higher concentration of Vitamin D in seminal plasma than in serum. A significant correlation was found between serum cortisol, seminal plasma and saliva C (r=0.90, p<0.001 and r=0.81, p=0.04, respectively. C in SP correlated with C in saliva (r=0.934, p=0.001) (Table 3). There was no correlation of the VD in serum and SP.

## Discussion

A new tool has been introduced in the Andrology Laboratory, the computer-assisted sperm analysis (CASA) systems. CASA systems have through advances in devices to capture the image from a microscope, huge increases in computational power concurrently with amazing reduction in size of computers, new computer languages, and updated/expanded software algorithms. It allows assessing the motility of individual spermatozoa, generating huge datasets. When carefully validated, CASA system provides important information for quality control and for the understanding of the diversity of sperm responses to changes in the microenvironment. The challenge is now focused in finding consistent biological meanings for this information, which allows an objective assessment of sperm kinetic that in our opinion is underused due to a lack of documented work to support its clinical value [20]. We standardized and validated a CASA system, SCA (Sperm Class Analyzer) for the parameters of sperm concentration and motility according to international standards, establishing that the proposed method meets the requirements for its use in the clinic [21]. We proved its use to evaluate patient's individual response to sperm motility improvement methods [22-23], nutraceutical supplementation [24], and varicocele surgery [25].

**Table 1:** Seminal plasma data on cortisol and Vitamin D in the studied population.

	N(n:43) (mean±SD)	P(n:48) (mean±SD)	p
Cortisol (µg/dL)	1.48±0.56	1.85±0.79	0.02
Vitamin D (ng/mL)	27.5±10.3	28.52±7.74	NS

**Table 2:** Correlation of cortisol and Vitamin D with seminal parameters.

	N + P (n:91)	P (n:48)	
	Cortisol	Cortisol	Vitamin D
Seminal volume (mL)	NS	r=-0.218, p=0.040	NS
Total sperm count (10 <sup>9</sup> /ejaculate)	r=-0.395, p<0.001	NS	NS
Sperm motility (%)	r=-0.241, p=0.03	NS	NS
Total motility (%)	NS	NS	r=0.350, p=0.025
ALH (µm)	r=0.258, p=0.022	NS	NS
BCF (Hz)	NS	r=0.318, p=0.04	NS

While considering male natural fertility, sperm motility plays a crucial role. Asthenozoospermia is the nomenclature related to a semen quality where the percentage of progressively motile spermatozoa is below the lower reference limit (32%) (WHO-2010). The Computer Assisted Sperm Analysis allows to better characterizing the different patterns of movements globally named asthenozoospermia.

Chronic stress has been associated with decreased semen quality but the mechanisms have not been elucidated. It is not known whether cortisol, the major stress hormone in humans, can act directly via receptors in the testis. On the other hand, experimental studies support a beneficial effect of Vitamin D on male fertility, by modulating hormone production through genomic and non-genomic actions, and, particularly, by improving semen quality essentially through non-genomic actions.

The expression of the Vitamin D receptor (VDR) and its metabolizing enzymes 25-alpha-hydroxylase (CYP2R1 or CYP27A1), 1-alpha-hydroxylase (CYP27B1) and 24 alpha -hydroxylase (CYP24A1) are present in the germ cells at spermatogenesis and persist in mature spermatozoa during their transit through the ejaculatory duct [26].

This findings support its probable rol in spermatogenesis and sperm maturation. VDR forms a heterodimer with the retinoid receptor (RXR) binding Vitamin D response elements (VDRE), thus regulating several transcription genes involved in mitosis, differentiation and apoptosis [27]. The partial nuclear expression of VDR in spermatogonia suggest a genomic action. VD can also induce a rapid non-genomic action, which is mediated by membrane or cytoplasmic VDR acting through second messengers [28-30]. The cellular response to VD does not only depend on the expression of VDR but also depends on the presence of activation of VD and inactivating enzymes.

It is important to note that spermatogonia expresses VDR, CYP27B1 and CYP24A1 but not CYP2R1 or CYP27A1 while spermatid and mature sperm being behind the blood-testicular barrier express them all. Spermatogonia is in better contact to blood and in this way has an easier access to the circulating 25 (OH) D3 [31].

**Table 3:** Relation of serum, saliva and seminal plasma cortisol.

	Saliva	Seminal plasma
Serum	r=0.81, p=0.04	r=0.90, p<0.001
Saliva		r=0.934, p=0.001

In addition to its role in cell cycle control, VD may trigger in a genomic way VD-dependent calcium transporters (TRPV5, TRPV6), calcium pump (PMCA), calbindin and calmodulin synthesis, all of them of great importance to assure sperm function [32-33].

The kidney and the ejaculatory tract have a common embryological origin, both of them derived from mesoderm. The isoosmotic reuptake of estrogen-dependent water and electrolytes in rete testis resembles the iso-osmotic (non-estrogen-dependent) reabsorption that takes place in the proximal tubules of the kidney [34].

Vitamin D in the ejaculatory tract may be involved in calcium transport as it is in the kidney and intestine [35]. This hypothesis is supported by the high content of calcium found in epididymal fluid, seminal vesicles and prostate. VDR and metabolizing enzymes are also expressed in the luminal vesicles of the epididymal caput/corpus they are excreted in the lumen of the ejaculatory tract therefore. Production of Vit D by the epididymis may explain the higher values found by our group in seminal plasma in regard to serum. The direct correlation between total motility and Vit D in the pathological samples may be thought as a compensatory action of the epididymis in cases of altered spermatogenesis. It is well known that ejaculated sperm are genetically silent [36]. It is speculated that in the mature spermatozoon co-express VDR and the metabolizing enzymes in the post-acrosomal/neck and middle part so that the activated Vitmine D can have a non-genomic action on the sperm. It has been shown that VD in small concentrations prolongs the survival of sperm ejaculates, modulating the composition of cholesterol and inducing phosphorylation of selected proteins [37].

According to our data either saliva and seminal plasma cortisol appear to be ultrafiltrates from serum. Cortisol has an inverse correlation with sperm count and total motility. It seems to have a direct effect on spermatogenesis, imprinting a particular type of kinetic, characterized by high energy but low progressiveness. This finding explain the positive correlation found between cortisol and ALH in all the samples and the positive correlation of cortisol and BCF in the pathological group. In this way we are characterizing a particular type of asthenozoospermia, highlighting one probable molecular mechanism involved in the process where a chemical substance, like cortisol produces biophysical changes, namely sperm kinetic. More generally speaking we are giving a new tool to understand how psychosocial stress may affect male fertility.

We conclude that cortisol would inversely affect sperm count and motility, imprinting a particular kinetics, VD acts only in the pathological samples probably due to a non genomic effect. Cortisol in seminal plasma as in saliva would be an ultra-filtrate of serum, while there might be local production of VD. The association found in other pathologies seem not to be resembled in seminal plasma probably due to local production of VD.

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## Author Contributions

**Mercedes Norma Pugliese:** 1) Substantial contributions to conceptions and design, acquisition of data, analysis and interpretation of data; 2) Drafting the article and revising it critically for important intellectual content; 3) Final approval of the version to be published.

**Gonzalez Diego:** 1) Analysis and interpretation of data; 2) Drafting the article and revising it critically for important intellectual content; 3) Final approval of the version to be published.

**Juan José Jamardo:** 1) Acquisition, analysis and interpretation of data; 2) Final approval of the version to be published.

**Ariagno Julia Irene:** 1) Analysis and interpretation of data; 2) Revising it critically for important intellectual content; 3) final approval of the version to be published.

**Repetto Herberto:** 1) Acquisition, analysis and interpretation of data; 2) revising it critically for important intellectual content; 3) final approval of the version to be published.

**Jacobsen Dario:** 1) Acquisition and interpretation of data; 2) revising it critically for important intellectual content; 3) final approval of the version to be published.

**Repetto Martin:** 1) Acquisition of data, analysis and interpretation of data; 2) Revising it critically for important intellectual content; 3) Final approval of the version to be published.

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**Mendeluk Gabriela Ruth:** 1) Substantial contributions to conceptions and design, acquisition of data, analysis and interpretation of data; 2) Drafting the article and revising it critically for important intellectual content; 3) Final approval of the version to be published.

## References

- Punab M, O Poolamets, P Paju, V Vihljajev, K Pomm, R Ladva, et al. Causes of male infertility: a 9-year prospective monocentre study on 1737 patients with reduced total sperm counts. *Hum Reprod.* 2017; 32: 18-31.
- Sikka SC, Hellstrom Wayne JG. Current updates on laboratory techniques for the diagnosis of male reproductive failure. *Asian Journal of Andrology.* 2016; 18: 392-401.
- Wang Christina, Swerdloff Ronald S. Limitations of Semen Analysis as a Test of Male Fertility and Anticipated Needs from Newer Tests. *Fertil Steril.* 2014; 102: 1502-1507.
- Rowe PJ, Compaire FH, Hargreave TB, Mellows HJ. WHO Manual for Standard Investigation and Diagnosis of the Infertile Couple. Cambridge, U.K. Cambridge University Press. 1993.
- Sharlip ID, Jarow JP, Belker AM, Lipshultz LI, Sigman M, Thomas AJ, et al. Best practice policies for male infertility. *Fertil Steril.* 2002; 77: 873-882.
- Hellhammer D, Wust S, Kudielka B. Salivary cortisol as a biomarker in stress research. *Psychoneuroendocrinology.* 2009; 34: 163-171.
- Aflakseir A, Zarei M. Association between Coping Strategies and Infertility Stress among a Group of Women with Fertility Problem in Shiraz, Iran. *J Reprod Infertil.* 2013; 14: 202-206.
- Boivin J, Griffiths E, Venetis CA. Emotional distress in infertile women and failure of assisted reproductive technologies: meta-analysis of prospective psychosocial studies. *BMJ.* 2011; 342: d223.
- Catherino WH. Stress relief to augment fertility: the pressure mounts. *Fertil Steril.* 2011; 95: 2462-2463.
- Matthiesen SM, Frederiksen Y, Ingerslev HJ, Zachariae R. Stress, distress and outcome of assisted reproductive technology (ART): a meta-analysis. *Hum Reprod.* 2011; 26: 2763-2776.
- Chrousos GP, Torpy DJ, Gold PW. Interactions between the hypothalamic-pituitary-adrenal axis and the female reproductive system: clinical implications. *Ann Intern Med.* 1998; 129: 229-240.
- World Health Organization: WHO Laboratory Manual for the Examination of Human Semen 5<sup>th</sup> ed Geneva, Switzerland: WHO Press. 2010.
- Irvine DS. Epidemiology and aetiology of male infertility. *Hum Reprod.* 1998; 13: 33-44.
- Curi SM, Ariagno JI, Chenlo PH, Mendeluk GR, Pugliese MN, Sardi Segovia LM, et al. Asthenozoospermia: Analysis of a large population. *Arch Androl.* 2003; 49: 343-349.
- Mendeluk GR, Ariagno JI, Repetto H, Jacobsen D, Gonzalez D, Repetto EM, et al. Psychosocial Stress and Asthenozoospermia: Case Report. *Austin Andrology.* 2017; 2: 1018.
- Blomberg Jensen M, Nielsen JE, Jorgensen A, Rajpert-De Meyts E, Moberg Kristensen D, Jorgensen N, et al. Vitamin D receptor and Vitamin D metabolizing enzymes are expressed in the human reproductive tract. *Hum Reprod.* 2010; 25: 1303-1311.
- Blomberg Jensen Martin. Vitamin D metabolism, sex hormones, and male reproductive function. *Reproduction.* 2012; 144: 135-152.
- Fabre B, Grosman H, Gonzalez D, Machulsky NF, Repetto EM, Mesch V, et al. Prostate Cancer, High Cortisol Levels and Complex Hormonal Interaction. *Asian Pac J Cancer Prev.* 2016; 17: 3167-3171.
- Fleet James C. Molecular Actions of Vitamin D Contributing to Cancer Prevention. *Mol Aspects Med.* 2008; 29: 388-396.
- Samplaski MK, Agarwal A, Sharma R, Sabanegh E. New generation of diagnostic tests for infertility: review of specialized semen tests. *Int J Urol.* 2010; 17: 839-847.
- Chenlo PH, Ariagno JI, Pugliese MN, Repetto H, Sardi LM, Mendeluk GR, et al. Study of human semen: implementation of an objective method. *Acta Bioquím Clín Latinoam.* 2013; 47: 61-69.
- Mendeluk GR, Chenlo PH, Sardi-Segovia M, Curi S, Ariagno J, Repetto H, et al. Usefulness of pentoxifylline to improve semen quality. *Fertil Steril.* 2010; 94: e28.
- Mendeluk GR, Rosales M. Thyroxine is useful to improve sperm motility. *Int J Fertil Steril.* 2016; 10: 208-214.
- Mendeluk GR, Cohen MI, Ferreri C, Chatgililoglu C. Nutrition and Reproductive Health: Sperm versus Erythrocyte Lipidomic Profile and  $\omega$ -3 Intake. *J Nutr Metab.* 2015; 2015: 670526.
- Ariagno JI, Mendeluk GR, Furlan MJ, Sardi M, Chenlo P, Curi S, et al. Computer aided sperm analysis, a useful tool to evaluate patient's response to varicoectomy. *Asian J Androl.* 2017; 19: 449-452.
- Blomberg Jensen Martin, Nielsen John E, Jørgensen Anne, Rajpert-De Meyts Ewa, Moberg Kristensen David, Jørgensen Niels, et al. Vitamin D receptor and Vitamin D metabolizing enzymes are expressed in the human male reproductive tract. *Hum Reprod.* 2010; 25: 1303-1311.
- Fleet James C. Rapid, Membrane-Initiated Actions of 1,25 DihydroxyVitamin D: What Are They and What Do They Mean? *JN the Journal of Nutrition.*

- 2004; 134: 12 3215-3218.
28. Norman Anthony W. Mini review: Vitamin D Receptor: New Assignments for an Already Busy Receptor *Endocrinology*. 2006; 147: 5542–5548.
29. The Vitamin D Receptor Is Present in Caveolae-Enriched Plasma Membranes and Binds 1,25(OH)<sub>2</sub>- Vitamin D<sub>3</sub> *in Vivo* and *in Vitro*. *Mol Endocrinol*. 2004; 18: 2660–2671.
30. Corbett Sean T, Hill Oya, Nangia Aay K. Vitamin D receptor found in human sperm. *Urology*. 2006; 68: 1345–1349.
31. Blomberg Jensen M. Vitamin D and male reproduction. *Nat Rev Endocrinol*. 2014; 10: 175-186.
32. Schoeber JPH, Hoenderop JGJ, Bindels RJM. Concerted action of associated proteins in the regulation of TRPV5 and TRPV6. *Biochemical Society Transactions*. 2007; 35: 115-119.
33. Inpanbutr N, Reiwig JD, Bacon WL, Slemmons RD, Iacopino AM. Effect of Vitamin D on Testicular CaBP28k expression and serum testosterone of chickens. *Biol Reprod*. 1996; 54: 242-248.
34. Clulow J, Jones RC and Hansen L. A Micropuncture and cannulation studies of fluid composition and transport in the ductuli efferentes testis of the rat: comparisons with the homologous metanephric proximal tubule. *Experimental Physiology*. 1994; 79: 915-928.
35. Blomberg Jensen M, Andersen CB, Nielsen JE, Bagi P, Jørgensen A, Leffers H. Expression of the Vitamin D receptor, the 25-hydroxylases, the 1-hydroxylase and the 24-hydroxylase in normal human kidney and in renal clear cell cancer. *J Steroid Biochem Mol Biol*. 2010; 121: 376-382.
36. Isabelle Galeraud-Denis, Sophie Lambard, Serge Carreau. Relationship between chromatin organization, mRNAs profile and human male gamete quality. *Asian J Androl*. 2007; 9: 587–592.
37. Áquila S, Guido C, Middea E, Perrotta I. Human male gamete endocrinology: 1 $\alpha$ , 25-dihydroxyVitamin D<sub>3</sub> (1,25(OH)<sub>2</sub>D<sub>3</sub>) regulates different aspects of human sperm biology and metabolism. *Reproductive Biology and Endocrinology*. 2009; 7: 140-153.