Tryptophan Catabolism to Serotonin and Kynurenine in Women Undergoing *in-vitro* Fertilization

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Summary

This cross-sectional clinical study was designed to explore the impact of tryptophan-kynurenine and tryptophan-serotonin (5-HT) pathways on reproductive performance during in vitro fertilization (IVF). Paired serum and follicular fluid (FF) samples were obtained from 64 consecutive IVF patients. The analysis was done by using LC-MS/MS. Ovarian hyperstimulation resulted in decreased serum tryptophan ($p<0.004$), 5-HT ($p<0.049$) and kynurenine ($p<0.001$). FF levels of tryptophan ($R=0.245$, $p<0.051$), kynurenine ($R=0.556$, $p<0.001$) and 5-HT ($R=0.523$, $p<0.001$) were positively related to their respective serum levels. Clinical pregnancy was associated with higher serum 5-HT ($p<0.045$) and FF 5-HT ($p<0.020$) and lower kynurenine to 5-HT ratio ($p<0.024$). Chemical pregnancy was also positively related to FF 5-HT ($R=0.362$, $p<0.024$). Moreover, there was a direct relationship of the number of mature oocytes to the FF 5-HT ($R=0.363$, $p<0.020$) but it was inversely related to FF tryptophan to 5-HT and FF kynurenine to 5-HT ratios ($R=-0.389$, $p<0.016$ and $R=-0.337$, $p<0.036$, respectively). Multivariate logistic regression revealed that the number of mature oocytes was significantly influenced by FF 5-HT ($\beta=0.473$, $p<0.001$). In IVF patients ovarian hyperstimulation results in a reduction of the availability of tryptophan to catabolic pathways to kynurenine and 5-HT. Outcome measures improved significantly when 5-HT predominated over kynurenine.

Key words

in vitro Fertilization • Tryptophan • Kynurenine • Serotonin • Oocyte maturation

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Introduction

The essential amino acid tryptophan acts as a precursor to various metabolic pathways that result in the production of proteins, serotonin (5-HT) and kynurenines. The major non-protein route for oxidative degradation of tryptophan (95 %) is the formation of kynurenine and downstream metabolites, collectively referred to as kynurenines. (Fig. 1) These biologically active compounds have been shown to be implicated in several conditions including pregnancy-related immune tolerance and inflammatory disorders (Wolf *et al.* 1970, Chen and Guillemin 2009, Stone *et al.* 2013).

Two enzymes initiate tryptophan catabolism; the hepatic tryptophan-2,3-dioxygenase (TDO) that is induced by glucocorticoids and tryptophan and inhibited by progesterone and estrogen, as well as the indoleamine-2,3-dioxygenase (IDO) which is present in a variety of cell types including macrophages and monocytes. It is up-regulated by certain cytokines and inflammatory molecules, but its most potent stimulant is interferon gamma (IFN-γ) (Sainio *et al.* 1996, Badawy 2015). Tryptophan depletion has anti-proliferative and apoptotic

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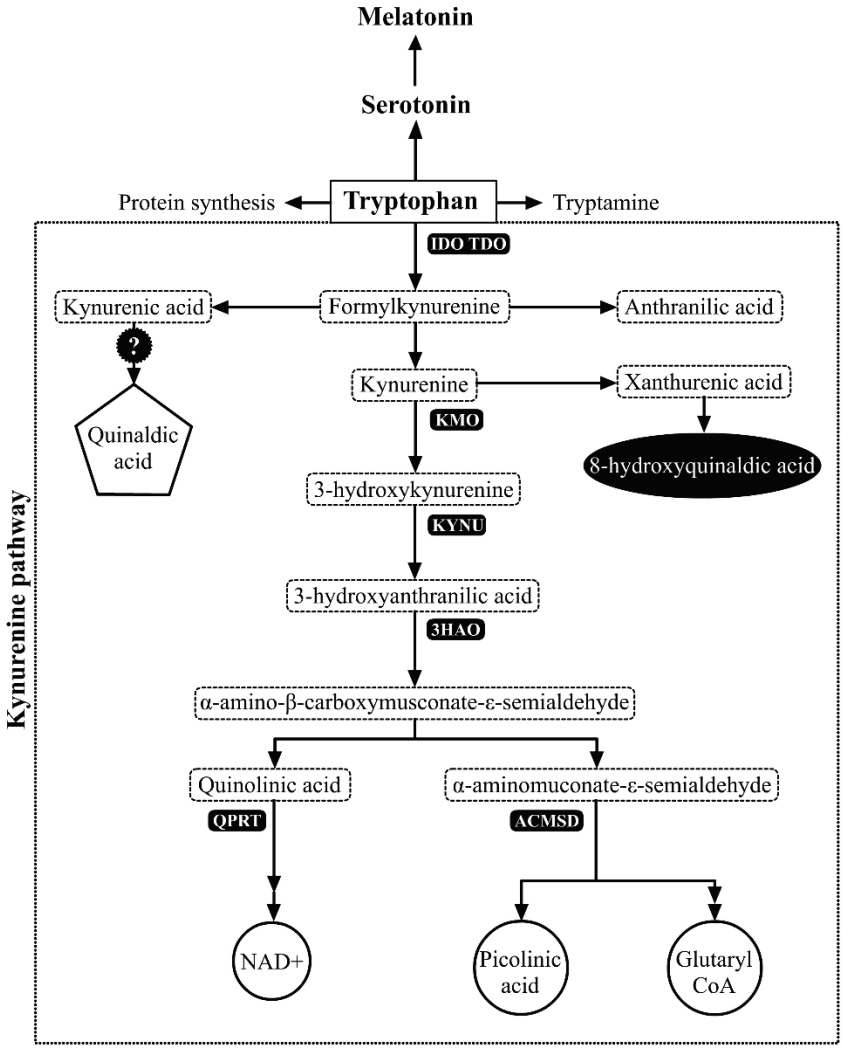

Fig. 1. Tryptophan catabolism with particular reference to the kynurenine pathway.
Abbreviations of the key enzymes involved in the kynurenine pathway:
IDO: Indoleamine 2,3 – dioxygenase
TDO: Tryptophane 2,3 – dioxygenase
KYNU: Kynureninase
KATs: Kynurenine aminotransferases
KMO: Kynurenine 3-monooxygenase
3HAO: 3-hydroxyanthranilic acid oxygenase
ACMSD: Aminocarboxymuconate-semialdehyde decarboxylase
QPRT: Quinolinic acid phosphoribosyltransferase

effect on T-cells (Munn *et al.* 1998, Munn *et al.* 1999, Lee *et al.* 2002). The low tryptophan levels are accompanied by elevation of kynurenines and by a subsequent reduction of 5-HT synthesis (Wichers *et al.* 2005).

In this regard it is to be stressed that serotoninergic regulation of the hypothalamic-pituitary-gonadal axis has been established and evidence has been provided for the role of 5-HT in female reproduction, particularly in the local intraovarian regulation (Kiss and Halasz 1985, Bodis *et al.* 1993, Li and Pelletier 1995, Hery *et al.* 1997). It is conceivable, therefore, that the activation of the tryptophan-kynurenine pathway and the simultaneous decrease of tryptophan bioavailability for 5-HT synthesis may have negative impact on reproductive performance. In support of this possibility the presence of tryptophan hydroxylase (the enzyme for 5-HT synthesis), 5-HT, its receptor and the 5-HT transporter has been documented in oocytes and preimplantation embryos suggesting that the paracrine/autocrine serotoninergic networks are functional already in the earliest embryonic development (Vesela *et al.* 2003, Il'kova *et al.* 2004, Amireault and Dube 2005a, Amireault and Dube 2005b).

In addition to 5-HT, kynurenines have also been demonstrated to be essential elements of reproduction by providing immune protection for implantation and for embryonic/fetal development (Mellor and Munn 2001, Kudo *et al.* 2004a, Groebner *et al.* 2011a, Grozdics *et al.* 2014, Badawy 2015).

On the basis of these observations it is relevant to assume that the production of 5-HT and kynurenines should be kept in balance and the shift of tryptophan catabolism either to 5-HT or to kynurenine pathway may compromise the success rate of fertilization.

The possible involvement of major metabolic hormones including insulin, leptin, adiponectin, resistin and ghrelin in the control of tryptophan catabolism, ovarian function and fertilization outcome has also been studied in IVF patients (Le Floc' *et al.* 2011, Várnagy *et al.* 2013, Dafopoulos *et al.* 2016). Their conflicting

results, however, warrant further studies to draw definitive conclusion on the diagnostic and/or therapeutic potential of these hormones in endocrine, - paracrine, - or autocrine regulation of ovarian function.

In order to get information about the role of tryptophan metabolism in women undergoing in vitro fertilization (IVF) the present study was designed a) to explore the response pattern of tryptophan, 5-HT and kynurenine to ovarian hyperstimulation, b) to assess the relationship between serum and follicular fluid (FF) levels of these hormones c) to establish the effects of 5-HT and kynurenine individually or in combination on reproductive performance (number of mature oocytes and embryos, chemical and clinical pregnancy) and d) to analyse the data of women with endometriosis separately as these patients are at particularly high risk for IVF failure.

Methods

Patients

This single centre, cross-sectional clinical study was performed between September 1 and October 1, 2015 and September 1 and November 1 2018 in the Assisted Reproduction Unit, Department of Obstetrics and Gynaecology, University of Pécs, Hungary. In these periods we started 64 unselected IVF cycles and made transvaginal ultrasound-guided aspiration of FF. The clinical parameters of the patients are given in Table 1.

All patients were on normal, unrestricted diet, neither of them had metabolic, – endocrine, – cardiovascular, – renal, – or psychiatric diseases so they did not receive drug therapy that might interfere with tryptophan metabolism.

The study was reviewed and approved by the Human Reproduction Committee of the Hungarian Medical Research Council (5273-2/2012/EHR). Signed informed consent was obtained from all patients who participated in the study. The investigation conforms to the principles outlined in the Declaration of Helsinki.

Protocols

The protocol of controlled ovarian hyperstimulation, the collection of serum and follicular fluid and fertilization were presented in our previous publication (Várnagy *et al.* 2018).

Laboratory measurements

The analysis of tryptophan, kynurenine, and 5-HT is based on phenylisothyocyanate (PITC) derivation in the presence of isotope labelled internal standards using liquid chromatography–mass spectrometry/mass spectrometry (LC-MS/MS) in the multiple reaction monitoring detection mode (tryptophan-d8 348.2/195.2, tryptophan 340.2/188.2, kynurenine-d6 350.2/151.2, kynurenine 344.2/146.2, d4-serotonin 316.3/164.2, serotonin 312.3/160.2). The method allows the simultaneous quantification using a 4000 QTrap mass spectrometer (SCIEX, Darmstadt, Germany) with electrospray ionization. The HPLC system consisted of a Shimadzu CBM-20A command module, two LC-20AD pumps, and a Shimadzu SIL-20AC-HT autosampler.

Table 1. Clinical characteristics of the patients studied

Characteristics	**n=64**
Age - yr	34.9±5.0
Nulligravid - n (%)	55 (85.9)
Nulliparous - n (%)	53 (82.8)
Duration of infertility - yr	3.9 ± 2.3
Body-mass index - kg/m^2	22.9±2.9
Cause of infertility - n (%)	
Poor semen quality	22 (34.4)
Tubal	16 (25.0)
Endometriosis	18 (28.1)
Other female	4 (6.3)
Combined male-female	1 (1.6)
Unexplained	3 (4.7)
No. of stimulation procedures initiated before	
Cycle 0	31
Cycle 1	16
Cycle 2	7
Cycle 3	7
Cycle 4	3
Serum estradiol - pmol/l	2593.3±3913.3
Total dose of gonadotropin - IU	2012.8 ± 621.4
Duration of stimulation - days	11.2 ± 2.8
No. of matured oocytes (metaphasis II)	6.33±4.72
No. of Grade 1 embryos	3.50±2.64
No. of transferred embryos (fresh only)	1.6 ± 0.8
hCG on day 12 - IU	318.4±715.2
No. of chemical pregnancies - n (%)	19 (29.7)
No. of clinical pregnancies - n (%)	18 (28.1)

Statistical analysis

Statistical analyses were performed using the 22.0 software of the SPSS (SPSS Inc., Chicago, IL, USA). Normality of data distribution was tested by Kolmogorov-Smirnov test. Depending on distribution Student t-test or Mann-Whitney U-test were used to compare continuous variables. The association between two continuous variables was tested by using Spearman's or Pearson's correlation coefficients. Multiple linear or logistic regression models were used to identify the variables independently associated with IVF outcome parameters (number of oocytes, number of embryos, chemical/clinical pregnancy). Data were expressed as median, 25-75 % quartiles and a value of $p<0.05$ was considered statistically significant.

Results

Table 2 shows serum and FF levels of tryptophan, 5-HT and kynurenine in the whole patient population who underwent IVF and separately in the non-pregnant and pregnant groups. It can be seen that in response to ovarian hyperstimulation there was a significant decrease in serum tryptophan [56.71 (50.33;65.98) vs. 50.18 (45.60;57.94) μmol/l, $p\leq 0.001$] kynurenine [1.72 (1.35;2.29) vs. 1.43 (1.19; 1.71) μmol/l, $p\leq 0.001$], and serum 5-HT [0.70 (0.56;1.02) vs. 0.65 (0.42;0.83) μmol/l, $p=0.049$]. FF levels of tryptophan [39.30 (33.23;48.74) μmol/l, $p\leq 0.001$] and 5-HT [0.04 (0.02;0.10) μmol/l, $p\leq 0.001$] were markedly depressed, while FF kynurenine remained unchanged [1.50 (1.33;1.81) μmol/l] when compared with their corresponding serum levels obtained at the time of oocyte retrieval. As a consequence, the serum tryptophan to kynurenine ratio increased significantly ($p=0.011$), whereas the rise in the serum tryptophan to 5-HT and kynurenine to 5-HT ratios did not reach statistical significance. In FF the tryptophan to kynurenine ratio decreased significantly ($p\leq 0.001$), however, the tryptophan to 5-HT ($p\leq 0.001$) and kynurenine to 5-HT ratios ($p\leq 0.001$) proved to be markedly elevated. These changes in the derived ratios are indicative of a shift of tryptophan catabolism from 5-HT to kynurenine, particularly within the ovaries.

When patients who underwent successful IVF treatment and progressed to clinical pregnancy and delivery at term were compared with those who failed to become pregnant similar response pattern was seen after hyperstimulation in each serum and FF tryptophan, kynurenine and 5-HT. It is of note, however, that the pregnancy positive patients had significantly higher serum ($p=0.045$), and FF ($p=0.020$) 5-HT levels and a marked reduction in the kynurenine to 5-HT ratio ($p=0.024$), as compared to the pregnancy negative patients. These findings suggest that pregnancy is associated with the diversion of tryptophan catabolism from kynurenine to 5-HT pathway.

Significant positive correlations were seen between serum and FF levels for 5-HT ($R=0.523$, $p\leq 0.001$) and kynurenine ($R=0.556$, $p\leq 0.001$) but not for tryptophan ($R=0.244$, $p=0.061$) indicating that 5-HT and kynurenine mainly originate from the maternal circulation, therefore, their local ovarian production might be quite limited.

Univariate regression analysis of hormonal interactions revealed that tryptophan was significantly related to 5-HT ($R=0.390$, $p=0.002$) but not to kynurenine. Furthermore, 5-HT was inversely related to tryptophan to 5-HT ($R=-0.898$, $p\leq 0.001$) and kynurenine to 5-HT ($R=-0.773$, $p\leq 0.001$) ratios, whereas kynurenine was negatively associated with tryptophan to kynurenine ($R=-0.687$, $p\leq 0.001$) but positively with kynurenine to 5-HT ratios ($R=0.640$, $p\leq 0.001$).

Table 3 demonstrates the association of selected clinical and hormone parameters with the products of tryptophan catabolism. It is apparent that the age of the patients had negative impact on FF tryptophan ($R=-0.270$, $p=0.037$) and FF 5-HT ($R=-0.494$, $p=0.001$), and FF tryptophan to kynurenine ratio ($R=-0.270$, $p=0.037$), while positively affected FF ratios of tryptophan to 5-HT ($R=0.465$, $p=0.003$) and kynurenine to 5-HT ($R=0.382$, $p=0.005$). Serum and FF levels of kynurenine remained unaffected by age. Moreover, estradiol levels were positively related to serum tryptophan ($R=0.281$, $p=0025$) whereas there was an inverse relationship between FSH dosage and FF tryptophan ($R=-0.310$, $p=0.013$). All parameters measured remained unaffected by the number of IVF cycles.

Importantly, the number of mature oocytes was positively related to FF 5-HT ($R=0.363$, $p=0.020$) and inversely to FF tryptophan to 5-HT and FF kynurenine to 5-HT ratios ($R=-0.389$, $p=0.016$ and $R=-0.337$, $p=0.036$, respectively). FF 5-HT was also positively associated with serum hCG levels taken on day 12 that is considered as an index of chemical pregnancy ($R=0.362$ $p=0.024$). Using multivariate logistic regression analysis correction was made for potential confounders and only the number

Table 2. Serum and follicular fluid levels of tryptophan, 5-HT and kynurenine during IVF (n=64)

	tryptophan (µmol/l)			5-HT (µmol/l)			kynurenine (µmol/l)			tryptophan/ kynurenine ratio			tryptophan/ 5-HT ratio			kynurenine/ 5-HT ratio		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
All patients (n=64)																		
Median	56.71	**50.18**$^{\#}$	39.30	.70	**.65**$^{\#}$	.04	1.72	**1.43**$^{\#}$	1.50	30.85	**35.75**$^{\#}$	**26.73**$^{\#\#}$	76.59	86.78	**763.93**$^{\#\#}$	2.30	2.17	**40.17**$^{\#\#}$
25th	50.33	45.60	33.23	.56	.42	.02	1.35	1.19	1.33	24.95	28.59	20.05	57.62	61.28	484.40	1.50	1.59	13.64
75th	65.98	57.94	48.74	1.02	.83	.10	2.29	1.71	1.81	40.78	45.27	35.97	105.79	139.50	1790.00	3.88	4.01	96.64
Non-pregnant Group (n=46)																		
Median	57.65	51.65	39.80	.64	.65	.04	1.72	1.43	1.51	29.25	36.16	26.82	84.26	87.09	903.61	2.92	2.25	49.64
25th	50.63	45.17	32.10	.53	.42	.01	1.36	1.20	1.33	24.91	28.14	19.84	63.91	62.06	526.86	1.53	1.54	28.33
75th	67.45	61.15	48.71	.99	.81	.07	2.28	1.74	1.82	43.39	45.67	35.97	112.33	144.16	2269.34	4.17	4.39	116.50
Pregnant Group (n=18)																		
Median	52.66	48.93	39.30	**.83***	.67	**.08***	1.58	1.37	1.48	32.64	33.24	23.66	61.97	71.63	506.97	1.89	1.96	**15.44***
25th	46.08	45.09	34.40	.68	.36	.04	1.31	1.15	1.33	26.20	29.20	20.09	45.87	55.35	257.18	1.31	1.62	2.90
75th	65.56	51.85	49.15	1.10	.91	.53	2.67	1.68	1.82	37.50	40.33	37.50	83.99	208.92	1575.56	3.55	6.42	60.39

IVF = *in vitro* fertilization, 5-HT=Serotonin, No. 1, 2, and 3 designates serum samples before (1) and after ovarian hyperstimulation (2) and follicular fluid samples (3), * Symbol for inter-group differences, $^{*}p< 0.05$, $^{\#}$ Symbol for intra-group differences, $^{\#}p<0.05$, $^{\#\#}p<0.01$

Table 3. Clinical and laboratory parameters influencing plasma and follicular fluid levels of tryptophan, 5-HT and kynurenine as well as tryptophan to kynurenine, tryptophan to 5-HT and kynurenine to 5-HT ratios during IVF (n=64)

		tryptophan (µmol/l)			5-HT (µmol/l)			kynurenine (µmol/l)			tryptophan/ kynurenine ratio			tryptophan/ 5-HT ratio			kynurenine/ 5-HT ratio		
		1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Age	*R*	-.227	-.209	**-.270***	-.142	-.069	**-.494****	.228	.138	.080	**-.326****	-.239	**-.282***	.081	-.099	**.465****	**,266***	.049	**.466****
(year)	*p*	.071	.097	**.037**	.275	.605	**.001**	.070	.278	.540	**.009**	.057	**.033**	.533	.459	**.003**	**,039**	,716	**.003**
Cycles	*R*	.076	-.076	-.129	-.114	-.096	-.092	.025	.013	.094	.033	-.030	-.176	.131	.124	.090	,074	,071	,102
(N)	*p*	.551	.548	.327	.380	.472	.569	.847	.916	.471	.795	.816	.190	.315	.352	.591	,571	,594	,535
E2	*R*	.067	**.281***	.146	.099	.153	-.011	-.112	-.027	-.088	.191	.171	.148	-.061	-.040	-.024	-,141	-,130	,018
(pmol/l)	*p*	.600	**.025**	.265	.446	.252	.945	.379	.832	.501	.130	.177	.273	.642	.767	.885	,279	,331	,914
FSH dosage (U/l)	*R*	-.116	-.115	**-.310**	-.180	.034	-.286	.157	.163	.065	-.205	-.135	-.170	.122	-.095	.192	.231	.011	.254
	p	.359	.366	**.013***	.165	.798	.070	.215	.199	.616	.104	.286	.206	.350	.480	.249	.074	.932	.119
Oocytes	*R*	-.011	.130	.197	-.030	.027	.275	-.100	.066	-.039	.109	-.005	.169	.006	.070	-.315	- .009	.062	- .229
(n)	*p*	.934	.306	.132	.818	.843	.082	.433	.605	.768	.392	.968	.210	.961	.600	.054	.945	.643	.161
Mature oocytes	*R*	.015	.073	.193	.030	.052	**.363***	-.171	-.020	-.102	.190	.013	.227	-.046	.042	**-.389***	- .087	.032	**- .337***
(n)	*p*	.907	.565	.140	.816	.701	**.020**	.177	.875	.436	.133	.916	.089	.723	.753	**.016**	.504	.812	**.036**
Embryos	*R*	-.117	-.011	.148	.060	-.075	.285	-.197	.046	-.089	.139	-.095	.176	-.092	.126	-.300	- .130	.156	- .278
(n)	*p*	.359	.929	.260	.644	.578	.071	.118	.716	.494	.272	.456	.191	.481	.344	.068	.316	.244	.087
hCG	*R*	-.030	-.045	.153	.233	-.036	**.362***	-.136	-.093	.052	.075	.003	.080	-.220	.030	-.293	- .217	.041	- .322
(IU)	*p*	.815	.728	.252	.076	.794	**.024**	.293	.473	.693	.561	.985	.563	.095	.829	.083	.099	.763	.052

IVF = *in vitro* fertilization, 5-HT=Serotonin, E2=Estradiol, FSH=follicle-stimulating hormone, No. 1, 2, and 3 designates serum samples before (1) and after ovarian hyperstimulation (2) and follicular fluid samples (3), R=Correlation Coefficient, p=significance level, *p< 0.05, **p< 0.01

Table 4. Serum and follicular fluid levels of tryptophan, 5-HT and kynurenine during IVF (n=64)

	Tryptophan (µmol/l)			5-HT (µmol/l)			Kynurenine (µmol/l)			tryptophan/ kynurenine ratio			tryptophan/ 5-HT ratio			kynurenine/ 5-HT ratio		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Patients with endometriosis (n=18)																		
Median	55.48	51.00	38.70	0.67	0.65	0.04	1.79	1.43	1.50	28.54	35.63	23.96	79.20	86.74	767.86	2.92	2.18	42.00
25th	49.88	42.90	32.85	0.55	0.42	0.02	1.35	1.20	1.33	24.82	28.59	19.55	61.39	61.72	501.80	1.74	1.63	14.00
75th	65.87	57.63	46.72	1.00	0.80	0.09	2.34	1.72	1.80	40.63	44.39	35.79	106.77	109.00	1820.00	4.14	3.76	109.50
Patients without endometriosis (n=46)																		
Median	59.48	48.72	49.84*	0.92	0.53	0.09	1.57	1.39	1.53	38.75	39.19	32.80	58.89	110.70	349.92	1.56	1.93	24.39
25th	52.81	46.14	38.84	0.59	0.01	0.04	1.35	1.15	1.32	31.34	29.10	21.70	55.65	54.90	290.27	1.32	1.48	8.80
75th	68.83	65.31	62.23	1.20	0.91	0.11	1.74	1.62	1.88	46.10	45.75	37.44	91.53	3874.67	786.70	2.30	124.58	38.91

IVF = *in vitro* fertilization, 5-HT=Serotonin, No. 1, 2, and 3 designates serum samples before (1) and after ovarian hyperstimulation (2) and follicular fluid samples (3), * Symbol for inter-group differences, *p<0.05

of mature oocytes as dependent variable was significantly influenced by serum estradiol (β=0.352, p=0.013) and FF 5-HT (β=0.473, p≤0.001). It is of note, that all of the outcome measures we investigated proved to be independent of serum and FF tryptophan and kynurenine.

When patients with endometriosis were considered as a separate group and their results were compared with those of the rest of the patients FF tryptophan was markedly depressed in the endometriosis group [38.70 (32.85;46.72) vs. 49.84 (38.84;62.23) p=0.023]. No significant differences could be found between the two groups in the other indices of tryptophan catabolism (Table 4). The major clinical characteristics and outcome measures proved also to be similar.

Discussion

Our study demonstrated that in patients undergoing IVF serum tryptophan, kynurenine and 5-HT decreased significantly in response to controlled ovarian hyperstimulation indicating a reduction of tryptophan bioavailability for catabolism to 5-HT and to kynurenine. All these compounds could be detected in FF and were closely related to their respective serum levels suggesting that FF tryptophan, 5-HT and kynurenine mainly derive from the maternal circulation rather than from local ovarian production. Clinical pregnancy was associated with higher serum and FF 5-HT and lower kynurenine to 5-HT ratio, while chemical pregnancy was positively related to FF 5-HT. Moreover, there was a direct relationship of mature oocytes to FF 5-HT but it was inversely related to the FF tryptophan to 5-HT and to FF kynurenine to 5-HT ratios. These findings indicate that in our clinical setting both the tryptophan-kynurenine and the tryprophan-5-HT pathways are activated but there is a shift from kynurenine to 5-HT when successful pregnancy is achieved.

To our knowledge no studies have been performed to investigate simultaneously the function of tryptophan-kynurenine and tryptophan-5-HT pathways in women undergoing IVF. However, convincing evidence has been provided for the essential role of tryptophan catabolism to both 5-HT and kynurenine in implantation, early embryonic and fetal development. With this contention in line embryo viability has been shown to be enhanced through 5-HT signalling (Doherty *et al.* 2011) with the possible contribution of embryonal, placental and maternal 5-HT (Cote *et al.* 2007, Bonnin and Levitt 2012). The contribution of multifunctional 5-HT to the maturation and differentiation of oocytes had already been reviewed by Buznikov *et al.* (1996). On the other hand, TDO, IDO and subsequent enzymes of kynurenine pathway have been identified in placenta, decidua and in early conceptus (Suzuki *et al.* 2001, Kudo *et al.* 2004a, Kudo *et al.* 2004b, Ligam *et al.* 2005, Manuelpillai *et al.* 2005).

The interdependence of tryptophan-5-HT and tryptophan-kynurenine metabolic pathways during the peri-implantation period is supported by the observations of Groebner *et al.* (2011a). They reported that in bovine pregnancy the endometrium had increased IDO mRNA expression and elevated tissue kynurenine concentrations but markedly reduced l-tryptophan and 5-HT. These findings are suggestive of a shift to the kynurenine pathway which resulted in decreased number of CD45-positive leukocytes and provided a possible immunological mechanism to establish embryo tolerance in early pregnancy (Groebner *et al.* 2011a).

However, the immune protection of the embryo by kynurenine is a matter of debate. Maternal cytokine profile in the first trimester of pregnancy is characterized by the increase of growth factors and by the predominance of anti-inflammatory cytokines. The absence of inflammatory environment, therefore, does not require immunosuppression by kynurenines (Hannan *et al.* 2014, Holtan *et al.* 2015, Yue *et al.* 2015). The elevated tryptophan levels at this stage of pregnancy may be accounted for by progesterone and estrogen inhibition of TDO, rather than the low expression of functional IDO (Badawy 1988, Ftukijwatari *et al.* 2004, Ligam *et al.* 2005). The excess tryptophan in early pregnancy has been claimed to serve as a substrate for protein and 5-HT synthesis. As pregnancy progresses tryptophan availability is maintained, IDO expression is up-regulated and immunosuppressive kynurenines are generated (Schrocksnadel *et al.* 1996).

The involvement of kynurenines in the control of embryonic/fetal development is further substantiated by demonstrating that IDO inhibition with 1-methyltryptophan or deletion of IDO gene cause pregnancy complications and fetal compromise (Munn *et al.* 1998, Santillan *et al.* 2015). Additionally, prenatal inhibition of the conversion of kynurenine to kynurenic acid alters developmentally relevant processes including synaptic plasticity and protein expression in the rat hippocampus (Forrest *et al.* 2013).

It is also to be considered that placental enzymes generating kynurenine (IDO, TDO) can clean free radicals (Britan *et al.* 2006) and some kynurenine pathway metabolites have also been shown to effectively remove reactive oxygen species (Christen *et al.* 1990, Weiss *et al.* 2002). Consistent with these observations phosphorylated tryptophan and its catabolism to kynurenine may activate nuclear factors that mediate the expression of antioxidant proteins and may provide

protection against free radical damage thereby improving redox status and reproductive performance (Xu *et al.* 2018). The tryptophan/kynurenine-related enhanced oxidative defence appears to be of particular importance in endometriosis where oxidative stress prevails (Fabjan *et al.* 2018, Várnagy *et al.* 2018). In our patients with endometriosis, however, adaptive activation of this antioxidant system could not be observed.

In our IVF patients we failed to document the activation of tryptophan-kynurenine pathway over the trypophan-5-HT pathway and to provide evidence for the protective role of kynurenine or its downstream metabolites. The reason for our failure is not apparent, further studies using molecular biology approach are to be conducted to reveal the complex interactions of the relevant clinical, endocrine and metabolic factors.

The association between the developmental potential of preimplantation embryo and amino acid metabolism has been the matter of intensive research and exogenous amino acid supply has been proposed to enhance embryo viability (Groebner *et al.* 2011b, Drábková *et al.* 2016). In view of the ovarian hyperstimulation – induced reduction in maternal serum tryptophan and the shift of tryptophan catabolism from the kynurenine to 5-HT pathway it is tempting to suggest that dietary tryptophan supplement to the mothers may improve the IVF outcome. It is to be considered, however, that most dietary tryptophan (>95 %) is metabolised via kynurenine pathway and only a small fraction (<5 %) of ingested tryptophan is available for 5-HT synthesis (Wolf 1974). Although, some of the downstream metabolites of kynurenine have beneficial, curative properties, others proved to be toxic and increase oxidative stress (quinolinic acid, 3-hydroxykynurenine, 3-hydroxy-anthranilic acid, picolinic acid) (Le Floc', Otten and Merlot 2011, Sainio *et al.* 1996). With respect to these controversies excess tryptophan intake is not recommended until its mechanisms of action, efficiency and safety is not clearly established.

In conclusion, adequate tryptophan supply for the generation of 5-HT and kynurenines is essential for the success of reproduction. We assume that this holds true for IVF, although only 5-HT but not kynurenine in maternal sera and FF were associated with the number of oocytes and chemical or clinical pregnancies. These observations support the notion that the tryptophan- 5-HT pathway prevails over the tryptophan-kynurenine pathway when chemical/clinical pregnancy is achieved.

Study limitations

We included IVF patients with heterogeneous infertility diagnosis, therefore the selection bias cannot be excluded. Furthermore, there are several important metabolites along the kynurenine pathway that compose a network of interrelated bioactive compounds, so the measurement of the precursor kynurenine alone does not allow to explore the impact of the whole system on the IVF process.

Conflict of Interest

There is no conflict of interest.

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