The Effects of Thymus Peptides and Oxytocin on Motility and Vitality of In Vitro Cultivated Human Spermatozoa

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Abstract : *The thymus is, according to the contemporary opinion, a part of the hypothalamus-pituitary-thymus-gonadal axis and participates in the regulation of the endocrine reproductive functions in human organisms. Besides specific hormone synthesis, neurohormone substances are made in the subcapsular zone (oxytocin, vasopressin). In human seminal plasma of fertile males, high levels of thymic hormone thymosin α 1 have been found, which influence the maturation processes and germinal cells function. High concentrations of neurohormone oxytocin (OT) have been recorded in the male reproductive tract. The results of the investigations confirm the influence of human fetal thymus extract (FTH) and juvenile calf thymus extract (JCTH) on the motility and vitality of human sperm. These extracts, which beside other biologically active substances, contain thymosin α 1 and oxytocin, were added to human sperm as a medium addition for sperm washing (SWM); after that, the investigation of their number and some vitality parameters was done: motility (M), progressive motility (PM), velocity motility (VM) and velocity of the progressive motility (VPM). Preliminary results of these studies indicate that the thymic factors application can be of use in the investigation and clinical treatment of male infertility, as well as in in vitro fertilization (IVF) procedures. (Thai J Obstet Gynaecol 1991; 3:13-19.)*

Key words : human spermatozoa, motility, sperm washing medium (SWM), fetal thymus extract (FTH), juvenile calf thymus extract (JCTH), oxytocin

The thymus is an important organ of the immunologic and endocrinologic system of the human organism. It has been proved that it plays an important role in the reproductive function of female and male gonads. A series of peptide substances are produced in the thymus and hormone bioactivity is the subject of intensive investigations. The most important among them are: thymosin α 1, thymosin β 4 and thymulin [1,2]. In the

subcapsular zone of the human and animal thymus neurohormones oxytocin and vasopressin have been found in relatively high concentrations [3-5]. Hall and Mcgillis[6] noted in their investigations the thymus-pituitary-gonadal axis existence. Neonatal thymectomy of experimental animals leads to gonadal dysgenesis[7]. Findings of the significantly decreased thymosin ß 4 concentrations in postmenopausal females and in those with surgically removed ovaries are very interesting[8]. Of special importance are the investigations which confirmed the presence of significantly higher thymosin concentrations in seminal plasma of fertile males in relation to the group of infertile ones, that is the confirmation of thymosin α 1 and fertility correlation[9]. Knowing that the thymus can be of the utmost importance in clinical andrology and reproductive endocrinology, we started an investigation of the influence of the thymus peptides from the extracts of human and juvenile calf thymus, and of the oxytocin on spermatozoa in *in vitro* conditions.

Materials and Methods

In cooperation with the Clinic of Gynaecology and Obstetrics, University Clinical Center in Belgrade, Institute of Biology and Human Genetics in Belgrade and Institute for Immunology and Thymus Reseach in Bad Harzburg, Federal Republic of Germany, we studied the influence of bioactive peptides (and neuropeptides) of human fetal and juvenile calf thymus extracts, and of synthetic oxytocin on 50 semen samples in *in vitro* conditions. Semen analysis was made after ejaculation of liquefaction[10]. Sperm washing media containing a series of substances such as enzymes, amino acids, antibiotics, hormones, serum, etc., were used in the investigation. Spermatozoal washing was performed twice and then centrifugation on 300 g. All testings were done in an incubator with controlled temperature, humidity and gaseous content, under the same conditions[11]. Hemocytometry was used for separation of motile and nonmotile spermatozoa. Such a "swim up" procedure enables separation of highly motile spermatozoa from the ones with less satisfactory vital abilities although it has been found that this procedure is definitely harmful for a certain number of spermatozoa, and their number was decreased after the washing[12]. The following parameters were determined in washed semen: number of vital spermatozoa, motility (M%), progressive motility (PM%), velocity motility (VM μm/s) and velocity progressive motility (VPM μm/s).

For our investigtions we used a) standard medium, b) standard medium (10 ml) and juvenile calf peptide thymus extract (1.5 mg), c) standard medium (10 ml) and lyophylization preparation of human fetal thymus extract (1.5 mg), and d) standard medium (10 ml) and oxytocin (Syntocinon) 1 IU.

These parameters were determined in washed sperm after 1hr,

24hr, and 48hr and the samples labelled with (a.) represented the control group to the standard medium for cultivated sperm.

The obtained information was numerically expressed and analyzed by X^2 and Student t-test on an IBM computer.

Results

Spermatozoa counting was determined in 50 analyzed ejaculations of 50 patients after the use of standard medium for spermatozoa washing (cultivation). After an hour, their number was 35 ± 15 x 10^6 ml, while higher values were found in native preparation (before sperm washing, 45 ± 10 x 10^6 ml, Table 1, Figure 1). Spermatozoa count was somewhat lower in samples cultivated in media added with juvenile calf (JCTH) or human fetal thymus extract (FTH) or synthetic oxytocin (30 ± 9 x 10^6 ml, Table 1, Figure 1). The increase of spermatozoal motility was observed in the medium with oxytocin (M=56± 11%) as well as human fetal thymus extract (M=55 ± 11%) (Table 1, Figure 2). Progressive motility (PM) was of higher values with oxytocin application (40 ± 12%) than with FTH (32 ± 15%, Table 1, Figure 3). Velocity motility (VM) was the highest in samples cultivated with oxytocin (39 ± 6 μm/s) and FTH (39 ± 11 μm/s, Table 1, Figure 4). Statistical analysis indicates borderline significance of these values. Special attention was paid to the investigation of progressive velocity motility (VPM). We found no significant differences between VPM of spermatozoa cultivated in standard medium and in native sperm (47 ± 12 μm/s, 40 ± 8 μm/s, respectively), while the oxytocin initiated a significant increase of this parameter (49± 5 μm/s, $p<0.05$, Table 1, Figure 5).

The values of the investigated parameters decreased 24 hours after the cultivation. However, it is interesting to point out that the motility was significantly higher in samples with JCTH (20 ± 6% and FTH (17 ± 5%), than in those with standard medium (9 ± 7%) and oxytocin (5 ± 2%) ($p<0.05$, Table 1, Figure 2). Progressive motility (PM) had almost the same values in all of the media, except in FTH application, where it was somewhat increased (10 ± 4%, Table 1, Figure 3). Spermatozoa cultivation in the media with JCTH and FTH caused increased velocity motility (VM) in comparison to oxytocin and standard medium (Table 1, Figure 4). Velocity of the progressive motility (VPM) was higher in samples with JCTH and FTH (22 ± 10 μm/s and 25 ± 9 μm/s, respectively), than in those with standard medium and oxytocin (18 ± 12 μm/s and 15 ± 8 μm/s, Table 1, Figure 5).

The fact that the greatest spermatozoa count was recorded 48 hours after the cultivation in samples with JCTH (7 ± 1 x 10^6 ml), with significance of $p<0.05$, is of particular interest (Table 1). Motility was the highest in preparations with FTH and JCTH (7 ± 2% and 10 ± 4%), especially in

Table 1 Influence of medium ingredients on the main spermatozoa features

		ST	JCTH	FTH	OT
Number	0h	45 ± 10	45 ± 10	45 ± 10	45 ± 10
$\bar{X}$ ± SD	1h	35 ± 15	27 ± 16	30 ± 9	30 ± 9
(x 10^6 / ml)	24h	12 ± 6	8 ± 2	9 ± 3	2 ± 1
	48h	5 ± 2	7 ± 1	2 ± 2	2 ± 2
M	0h	46 ± 5	46 ± 5	46 ± 5	46 ± 5
$\bar{X}$ ± SD	1h	52 ± 12	50 ± 7	55 ± 11	56 ± 9
(%)	24h	9 ± 7	20 ± 6	17 ± 5	5 ± 2
	48h	5 ± 1	10 ± 4	7 ± 2	2 ± 1
PM	0h	27 ± 8	27 ± 8	27 ± 8	27 ± 8
$\bar{X}$ ± SD	1h	30 ± 15	29 ± 13	32 ± 15	40 ± 12
(%)	24h	6 ± 5	9 ± 3	10 ± 4	5 ± 1
	48h	7 ± 2	8 ± 2	9 ± 2	4 ± 1
UM	0h	31 ± 7	31 ± 7	31 ± 7	31 ± 7
$\bar{X}$ ± SD	1h	36 ± 10	35 ± 10	39 ± 11	39 ± 6
(μM/s)	24h	11 ± 4	15 ± 6	20 ± 7	11 ± 5
	48h	11 ± 5	20 ± 7	19 ± 2	15 ± 7
UPM	0h	40 ± 8	40 ± 8	40 ± 8	40 ± 8
$\bar{X}$ + SD	1h	47 ± 12	45 ± 9	35 ± 7	49 ± 5
(μM/s)	24h	18 ± 12	22 ± 10	25 ± 9	15 ± 8
	48h	18 ± 9	25 ± 6	11 ± 6	15 ± 1

ST - Standard medium
FTH - Fetal thymus extract
Number - Number of spermatozoa
PM - Spermatozoa progressive motility
VPM - Spermatozoa velocity progressive motility
Oh - Before adding ingredients
1h - One hour after adding ingredients
JCTH - Juvenile calf thymus extract
OT - Oxytocin
M - Spermatozoa motility
VM - Spermatozoa velocity motility
24h - hours after adding ingredients
48h - hours after adding ingredients

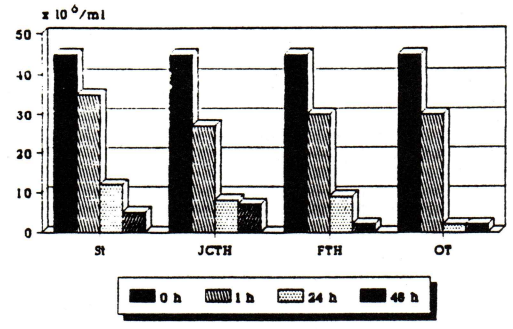

Fig. 1 Number of spermatozoa.

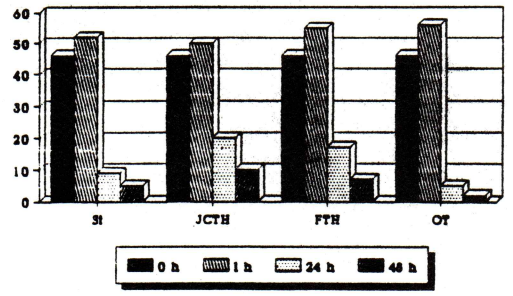

Fig. 2 Spermatozoa motility.

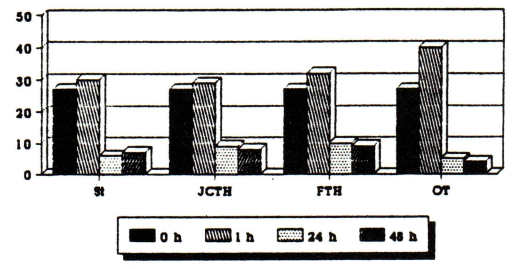

Fig. 3 Spermatozoa progressive motility.

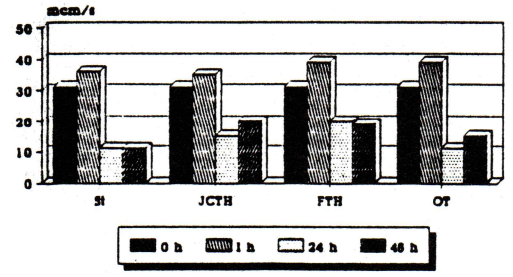

Fig. 4 Spermatozoa velocity motility.

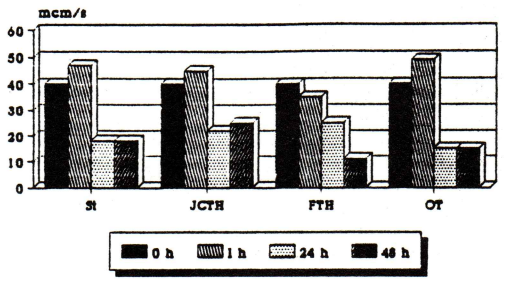

Fig. 5 Spermatozoa velocity progressive motility.

relation to the values in oxytocin media (2 ± 1%), (Table 1). PM and VM were of higher values with JCTH and FTH presence. VPM was statistically significantly higher in preparations treated with JCTH (25 ± 6 μm/s), than in other media ($p<0.05$) (Table 1, Figures 4, 5).

Discussion

Parameters of functioning or to be more precise of spermatozoal vitality investigated in this study are of crucial importance for *in vitro* and *in vivo* fertilization. Today, when we have knowledge of numerous physiological properties and constituents of seminal fluid in the procedures of spermatozoal preparation for intrauterine insemination (IUI), in vitro fertilization (IVF), gamete intra-Fallopian tubes transfer (GIFT), media containing different bioactive substances (amino acids, enzymes, antibiotics, nutritive factors, hormones, etc.) are used for "swim up" [13, 14, 15]. Recent studies proved high thymosin α 1 levels in seminal plasma, in correlation to the spermatozoal count, viability and

motility in fertile males, in comparison to infertile ones, having more prominently decreased parameters[9].

After standard medium usage for semen washing, spermatozoal count was of much lower value after 24hr and 48hr since only more vital ones remained (managed to survive). Global motility and velocity of the progressive motility were increased after an hour in relation to the values prior to incubation.

After spermatozoal cultivation in medium with juvenile calf thymus extract (JCTH) was added, a longer period of global motility maintenance was observed, especially of VPM, in respect to the spermatozoa in standard medium.

Addition of human fetal thymus extract stimulates global motility at first, then the progressive one, and also the spermatozoal velocity motility, in all of the testing time intervals. JCTH and FTH represent total thymus extracts, which, besides the specific thymus peptides, contain neuropeptides, such as oxytocin. Therefore, synthetic oxytocin addition in standard medium for spermatozoal washing (cultivation) causes characteristic changes. Significant increase of M, PM and VPM of the spermatozoa values occurred an hour after addition of a medium with oxytocin, while after 24hr and 48hr sudden decrease of spermatozoal vitality developed. Oxytocin stimulates intercelluar glucose oxidation initiating a prompt energetic potential release significant for cellular development and activity[17].

Investigations of Maggi et al[16] indicate that oxytocin receptors, which play a very complex role in human reproductive endocrinology, are present in the male genital tract.

Media for spermatozoal cultivation, which contain specific thymus peptides and neuropeptides, manifest influence on their motility and vitality in *in vitro* conditions, and is of great importance for future investigations in reproductive clinical endocrinology.

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