CLAIMS

1. Method for the investigation of the presence of viruses, chlamydia, parasites and other infectious pathogens intracellularly in spermatozoa, which method comprises the following steps:
   - A procedure for the
   - easing of the dense structure of the DNA of the sperm cells including spermatozoa,
   - direct or indirect intra-sperm cell immunofluorescence,
   - visualization and evaluation of results by flow cytometry,

   and which method is characterized by the fact that the step of easing of the dense structure of the DNA must necessarily take place before immunofluorescence.

2. Method according to Claim 1 wherein the step of visualization and evaluation of results by flow cyttometry comprises incubation of cell pellets with 7-aminoactinomycin D (7AAD) in WB in order to enable the discrimination between 1N and 2N cells.

3. Method according to any one of claims 1 or 2, which is used for the determination of the causes of infertility, of early pregnancy faillure, miscarriage or fetal loss and for the prevention of congenital infections or for the prevention of vertical transmission and also for the investigation of inflammation and infections of the male genital system.

4. Method according to any one of claims 1 to 3, which is used specifically for the investigation of one of the following infectious agents: Cytomegalovirus (CMV), Herpes Simplex Virus I (HSV I), Herpes Simplex Virus II (HSV II), Epstein Bar Virus (EBV), HHV6, HHV7, HHV8, Parvovirus 19, Hepatitis B Virus (HBV), Hepatitis C Virus (HCV), Coxsackie Virus, Human Immunodeficiency Viruses (HIV-1, HIV-2), Adeno-associated Virus (AAV), Rubella Virus, HPV, Chlamydia, Toxoplasma and Norovirus.

5. Method according any one of claims 1 to 4, which is used for the investigation for the presence of chlamydia in spermatozoa in combination with spermiogram and cultures using the same sample or on different samples.

6. Method according to any one of claims 1 to 5, where the easing of the very dense DNA structure of spermatozoa DNA is performed with DNA digestion.

7. Method according to claim 6, where DNA digestion is accomplished with an enzyme that breaks DNA.

8. Method according to any one of claims 6 or 7, where the enzyme that breaks DNA is DNase I.

9. Method according to any one of claims 1 to 8, where any appropriately labeled anti-antibody can be used for fluorescence.

10. Method according to claim 9, where for fluorescence any fluorochrome can be used, among which any one of the following: Fluorecein-5- isothiocyanate (FITC), aminomethylcoumarin Acetate (AMCA 350), 6,8-difluoro-7-hydroxycoumarin derivative (Marina Blue), Cascade Blue, Alexa fluor 405, 6,8-difluoro-7-hydroxycoumarin derivative (Pacific Blue), Alexa Fluor 430, Cascade Yellow, Alexa Fluor 488, phycoerythrin (PE), phycoerythrin Texas Red (PE-Texas Red), phycoerythrin-cyanin 5 (PE-Cy5) , peridinin chlorophyll protein (PerCP), peridinin chlorophyll protein –cyanin 5.5 (PerCP-Cy5.5), phycoerythrin-cyanin 7(PE-Cy7), Rhodamine TR, allophycocyanin (APC), ALexa Fluor 647, allophycocyanin cyanin 7 (APC-Cy7), BD APC-H7, Alexa Fluor 700.

11. Method according to any one of claims 1 to 10, where an extra step of external immunofluorescense is included.

12. Kit for the investigation of the presence of viruses, chlamydia, parasites and other pathogens inside spermatozoa, which comprises at least the following:

- a substance that can ease DNA of spermatozoan cells,
- one or more antibodies appropriate for the use against the infectious agents of interest.

13. Kit according to claim 12, where the DNA digestion substance is any DNA digestion enzyme.

CLAIMS

1. Method for the investigation of the presence of viruses, chlamydia, parasites and other infectious pathogens intracellularly in spermatozoa, which method comprises the following steps:
   - A procedure for the de-condensation or easing of the dense structure of the DNA of the sperm cells including spermatozoa,
   - direct or indirect intra-sperm cell immunofluorescence targeted towards said infectious pathogens,
   - visualization and evaluation of results by flow cytometry,

   and which method is characterized by the fact that the step of easing of the dense structure of the DNA must necessarily take place before immunofluorescence.

2. Method according to Claim 1 wherein the step of visualization and evaluation of results by flow cytometry comprises incubation of cell pellets with 7-aminoactinomycin D (7AAD) in wash buffer (WB) in order to enable the discrimination between 1N (aploid) and 2N (diploid) cells.

3. Method according to any one of claims 1 or 2, which is used for the determination of the causes of infertility, of early pregnancy failure miscarriage or fetal loss and for the prevention of congenital infections or for the prevention of vertical transmission and also for the investigation of inflammation and infections of the male genital system.

4. Method according to any one of claims 1 to 3, which is used specifically for the investigation of one of the following infectious agents: Cytomegalovirus (CMV), Herpes Simplex Virus I (HSV I), Herpes Simplex Virus II (HSV II), Epstein Bar Virus (EBV), Human Herpes Virus 6 (HHV6), Human Herpes Virus 7 (HHV7), Human Herpes Virus 8 (HHV8), Parvovirus 19, Hepatitis B Virus (HBV), Hepatitis C Virus (HCV), Coxsackie Virus, Human Immunodeficiency Viruses (HIV-1, HIV-2), Adeno-associated Virus (AAV), Rubella Virus, HPV, Chlamydia, Toxoplasma and Norovirus.

5. Method according any one of claims 1 to 4, which is used for the investigation for the presence of chlamydia in spermatozoa in combination with spermiogram and cultures using the same sample or on different samples.

6. Method according to any one of claims 1 to 5, where the easing of the very dense DNA structure of spermatozoa DNA is performed with DNA digestion.

7. Method according to claim 6, where DNA digestion is accomplished with an enzyme that breaks DNA.

8. Method according to any one of claims 6 or 7, where the enzyme that breaks DNA is DNase I.

9. Method according to any one of claims 1 to 8, where any appropriately labeled anti-antibody can be used for fluorescence.

10. Method according to claim 9, where for fluorescence any fluorochrome can be used, among which any one of the following: Fluorecein-5- isothiocyanate (FITC), aminomethylcoumarin Acetate (AMCA 350), 6,8-difluoro-7-hydroxycoumarin derivative (Marina Blue), Cascade Blue, Alexa fluor 405, 6,8-difluoro-7-hydroxycoumarin derivative (Pacific Blue), Alexa Fluor 430, Cascade Yellow, Alexa Fluor 488, phycoerythrin (PE), phycoerythrin Texas Red (PE-Texas Red), phycoerythrin-cyanin 5 (PE-Cy5) , peridinin chlorophyll protein (PerCP), peridinin chlorophyll protein –cyanin 5.5 (PerCP-Cy5.5), phycoerythrin-cyanin 7(PE-Cy7), Rhodamine TR, allophycocyanin (APC), ALexa Fluor 647, allophycocyanin cyanin 7 (APC-Cy7), BD APC-H7, Alexa Fluor 700.

11. Method according to any one of claims 1 to 10, where an extra step of external immunofluorescence is included.

12. Kit for the investigation of the presence of viruses, chlamydia, parasites and other pathogens inside spermatozoa, which comprises at least the following:

- a substance that can ease DNA of spermatozoan cells,
- one or more antibodies appropriate for the use against the infectious agents of interest.

13. Kit according to claim 12, where the DNA digestion substance is any DNA digestion enzyme.