

tissue, followed by approximating the remaining myometrium and serosa. However, this method may retain unexcised affected tissue, and thus result in an unsatisfactory post surgical prognosis such as being incapable of sustaining a normal pregnancy. Our proposed treatment for severe cases of adenomyosis involves wide complete excision of affected tissues to reduce post surgical dysmenorrhea, followed by a triple-flap reconstruction of the uterine wall to prevent ruptures in subsequent pregnancies.

#### Methods:

1. Resection and removal of all adenomyosis-affected myometrium: The affected tissue is vertically incised, to split the area to be excised in two, the incision is extended to the uterine cavity. The tissue to be excised is grasped and placed under tension with Martin forceps. The tissue is adequately dissected free with scissors, with care taken to retain a serosal flap with a layer of myometrium, as well as a medial flap containing both endometrium and myometrium. The tissue flaps, both medial and distal must be more than 5 mm in thickness to assure adequate material for the reconstruction of the uterine wall. It is essential to introduce an index finger into the uterine cavity to assure maintenance of an adequate medial flap thickness. Special care must be taken to prevent damage to the Fallopian tubes.

2. Reconstruction of the uterine cavity: Care must be taken to retain sufficient endometrium to allow reconstruction of an adequate uterine cavity. In cases of an over abundance of endometrial tissue, excess amounts must be removed to secure a more physiological uterine cavity.

3. Reconstruction of the uterine wall: Reconstruction of the middle portion of the uterine wall involves approximation of the myometrial musculature to ablate the space created by the excision of diseased tissue. The serosa including adequate myometrium is dissected free with a scalpel to form the third flap. The serosal or distal and third flap is then approximated to finish the reconstruction.

4. Hemostasis and application of hemostatic barriers for prevention of adhesion: The last step of this method is to apply TachoComb®, a Fibrin adhesive in sheet form, to the uterine surface for the control of oozing. The applied TachoComb® is firmly anchored and works as physical barriers, thus contributing to the reduction of post surgical adhesions.

#### Results:

Clinical post surgical evaluation was performed using the Visual Analog Scale (VAS) to assess dysmenorrhea and hypermenorrhea at 3, 6, 12, 24 months after surgery. We performed the procedures on 104 patients during the period between June 1998 and August 2008. Of the 26 women desired to conceive, 16 (61.5%) subsequently conceived. Of these, 4 women conceived spontaneously and 12 women conceived by in vitro fertilization and embryo transfer (IVF-ET). Two women who had IVF-ET experienced a spontaneous abortion; 14 went to term and all were delivered by elective Caesarean section. There were no cases of uterine complications to the pregnancies. The triple-flap reconstruction of the uterine wall following wide adequate excision of adenomyosis tissue in women with hypermenorrhea and/or dysmenorrhea resulted in a dramatic reduction in both menstrual cramping and menstrual flow volume post surgically and gave women chances to become pregnant.

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##### MILD OVARIAN STIMULATION IN ART AND ELECTIVE DOUBLE EMBRYO TRANSFER (E-DET). A PROSPECTIVE NON RANDOMIZED CONTROLLED TRIAL

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#### Introduction:

Mild ovarian stimulations in ART are being increasingly advocated as a means of achieving satisfactory live birth rates while minimizing severe side effects such as multiple pregnancies and ovarian hyperstimulation syndrome. They also attempt to decrease costs and increase availability, reduce abandoned embryos, and being friendlier, diminish patient drop out from treatment. To really accomplish their first task, fewer embryos should be transferred. We wanted to know if the approach of mild ovarian stimulation in conjunction with e-DET in good prognosis patients is appropriate enough to implement in a setting as ours.

#### Materials/Methods:

Seventy seven good prognosis patients (less than 35, FSH less than 8.5, non smoking, non obese) with different causes of infertility underwent a mild ovarian stimulation (150 FSHr and GnRH antagonist in fixed daily scheme) for ART (IVF or ICSI) and e-DET was performed in a 6 month period. As control group we included 53 patients of the same characteristics who, after extensive counselling, didn't accept to enter the trial and insisted, for diverse reasons, in their preference for conventional stimulation and 3 embryos transferred.

#### Results:

In the study group, the cancelation rate was 9%, average number of oocytes retrieved 6.4, the clinical PR 46%, implantation rate 30.25%, ongoing PR 36%, single PR 70%, double PR 30%. In the control group the cancelation rate was 3.7%, average number of oocytes retrieved 10.4, Clinical PR 45%, implantation rate 20.7%, ongoing PR 35%, single, double and triple PR were 70.83, 20.83 and 8.33% respectively.

#### Conclusions:

In selected good prognosis patients mild ovarian stimulation with FSHr and GnRH antagonist in conjunction with e-DET seems to be an effective and suitable approach that significantly reduces risks and keeps a good live birth rate similar to the one obtained with more aggressive regimes. This should be widely recommended to this kind of patients in an extensive counselling visit. The improvement on the implantation rate is probably due to the simpler approach closer to physiology which influences oocyte quality and endometrial receptivity. Although, it still produces a high number of double pregnancies and is a preliminary step to introduce e-SET in a private setting where patients have to afford the cost of ART and sometimes are not able to have several ART attempts.

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##### CLINICAL EXPERIENCE FOR THE TREATMENT OF MALE AUTOIMMUNE INFERTILITY WITH LONGIDAZA

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We carried out the treatment of 14 male with autoimmune infertility, positive detection rate of the mixed agglutinin reaction (MAR) test. All patients had concomitant diseases, such as chronic inflammatory reproductive tract diseases, hypogonadism, one- or two-sided varicocele, epididymis cysts,

the mean age was 33.29 years. The clinical trial was conducted in Ivanovo clinic-diagnostic center of municipal hospital No. 8. Statistical analysis was performed with Microsoft Excel v.12.0 and Statistica v6.1. Men applied to the hospital with infertility and diagnosed the autoimmune infertility (MAR%IgG and/or MAR%IgA  $\geq$  50%) were included in this trial. Spermogram results: the mean spermatozoa with adhesive antisperm antibodies (ASA) type IgG was 54%, and type IgA – 60%; mean spermatozoa A – 9%, mean spermatozoa B – 16%; total amount of spermatozoa in ejaculate A – 22.46 mil, total amount of spermatozoa in ejaculate B – 32.98 mil; mean spermatozoid speed was 8.83  $\mu$ m/s, mean viscosity – 3.63 cm.

All patients were treated with Longidaza. All the same indicators were evaluated after 3 months of treatment. The mean spermatozoa with ASA type IgG significantly decreased to 15% ( $p=0.0016$ ), type IgA – to 14% ( $p=0.001$ ). Mean spermatozoa A was 11%, B – 21%, A+B – 32%, total amount of spermatozoa in ejaculate A – 30.08 mil, total amount of spermatozoa in ejaculate B – 30.35 mil; mean spermatozoid speed was 9.67  $\mu$ m/s, mean viscosity – 3.18 cm. No side or adverse effects were registered by the patients during the treatment. Therefore, statistically-valid decrease of spermatozoa with ASA type IgG mean 51%, and type IgA – 37%. Qualitative and quantitative spermogram indicators improved, but not statistically-valid, apparently, this is connected with small sample size.

### 37 MORPHOLOGICAL FEATURES OF SPERMATOZOA (LIKE "HEAD-IN-TAIL" ANOMALY) AND "COMET ASSAY" FROM PATIENTS WITH DECLINED QUALITY OF SEMEN

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#### Introduction:

Morphological examination of semen is an important and reliable source of information about male fertility. The morphological criteria of semen quality have been standardized in recent years, elaborating the multiple anomalies index (MAI) which improved the spermatozoa examination, allowing us to elucidate anomalies which can affect spermatozoa function. One of the morphological anomalies of sperm is the so-called "head-in-tail" anomaly. The mechanism behind the "head-in-tail" anomaly is not clear, and the role of the morphological anomaly in male fertility impairment has been extensively discussed. The "comet assay" (single cell gel electrophoresis) was shown as the method of choice for evaluating the integrity of sperm DNA.

#### Patients and Methods:

Sperm analysis and "comet assay" were performed in 115 patients (average age 32 years, average abstinence time 5 days). The functional (motility, concentration, volume, velocity etc.) and morphological characteristics of sperm were accurately registered in each case. The morphology of one out of 200 spermatozoa was studied for the presence of anomalies. Our particular attention was directed at the results of the "comet assay" and the study of "head-in-tail" anomaly.

#### Results:

The study of sperm motility showed that it decreases along with the increase of morphological anomalies. The ejaculate concentration in patients with spermatozoa characterized by high MAI (more than 1.6) was slightly decreased. However, the difference between healthy donors (79.20 $\pm$ 6.23 mil. cells per ml) and patients with impaired sperm morphology (74.09 $\pm$ 17.41

mil. cells per ml) was found to be not significant ( $p > 0.05$ ). Higher frequency of morphological sperm anomalies was followed with slight increase in damaging the DNA integrity of spermatozoa (16 $\pm$ 2.9%). The DNA integrity of spermatozoa from healthy donors was 11 $\pm$ 3.5% ( $p > 0.05$ ). The anomaly "head-in-tail" was equal to 3.1 $\pm$ 1.4% and 5.8 $\pm$ 1.8% in sperm from healthy donors and patients with elevated frequency of morphological sperm anomalies of spermatozoa relatively, though the difference was insignificant ( $p > 0.05$ ).

### 38 H3S10P, PHOSPHORYLATION OF HISTONE H3 AT SER10 IN PREIMPLANTATION MOUSE EMBRYOS

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The nucleus undergoes impressive changes during the cell cycle and nuclear structure together with chromatin organization are both very important matters when it comes to gene regulation. Similarly, embryonic nuclei are under the influence of these dramatic changes. Therefore knowing the mechanisms behind these changes is fundamental in understanding the regulatory processes involved in development.

Our team previously showed that the dynamic of pericentromeric heterochromatin (the chromatin located at the periphery of the centromeres) is related to the developmental potential of embryos, probably through unknown mechanisms related to gene regulation. In particular, this seems to be the case after SCNT (somatic cell nuclear transfer). Therefore, finding a marker of this type of heterochromatin is a way of evaluating the embryo development rate after SCNT, as well as a method of improving it by manipulation of this specific marker.

We hypothesize that an epigenetic modification, phosphorylation of histone H3 at Serine 10 (H3S10P), known to correlate with chromatin condensation, could be a good marker of pericentromeric heterochromatin and that it could be used to evaluate reprogramming after SCNT. Indeed, in somatic cells, this epigenetic modification appears within the aggregates of pericentromeric heterochromatin called chromocenters at the end of interphase. During mitosis, this modification spreads throughout the chromosome arms, starting from prophase, and it is only at the end of anaphase, that histone H3 gets gradually dephosphorylated at Serine 10.

In this study we found that this epigenetic modification shows a different distribution pattern in mouse embryos. Using an antibody specific for this modification and high resolution microscopy, we have observed that, just after fertilization, H3S10P is already detected in early interphase on the heterochromatin rings around the Nuclear Precursor Bodies (NPB). During the first mitosis, H3S10P is seen on the chromosome arms and maintained upon the formation of the nuclei in the daughter cells. It is only at the late 2-cell stage, when the chromocenters appear for the first time, that H3S10P shows some similarities to the spatial distribution seen in somatic cells. At that stage, H3S10P is seen in the heterochromatin rings around the NPBs as well as in the chromocenters. It is only after the 4-cell stage that H3S10P acquires the same kinetic as is seen in somatic cells: H3S10P cannot be detected in the heterochromatin rings anymore and is only located in the chromocenters during late interphase, as well as on the mitotic chromosomes until telophase. This kinetic is maintained throughout the next cell cycles until the blastocyst stage.

We believe that such a unique phosphorylation kinetic of histone H3 at Serine 10 may play specific roles during mammalian embryonic development and intend to use it as a marker to evaluate the embryonic development of reconstructed embryos after SCNT. Keeping in mind that after this procedure pericentric