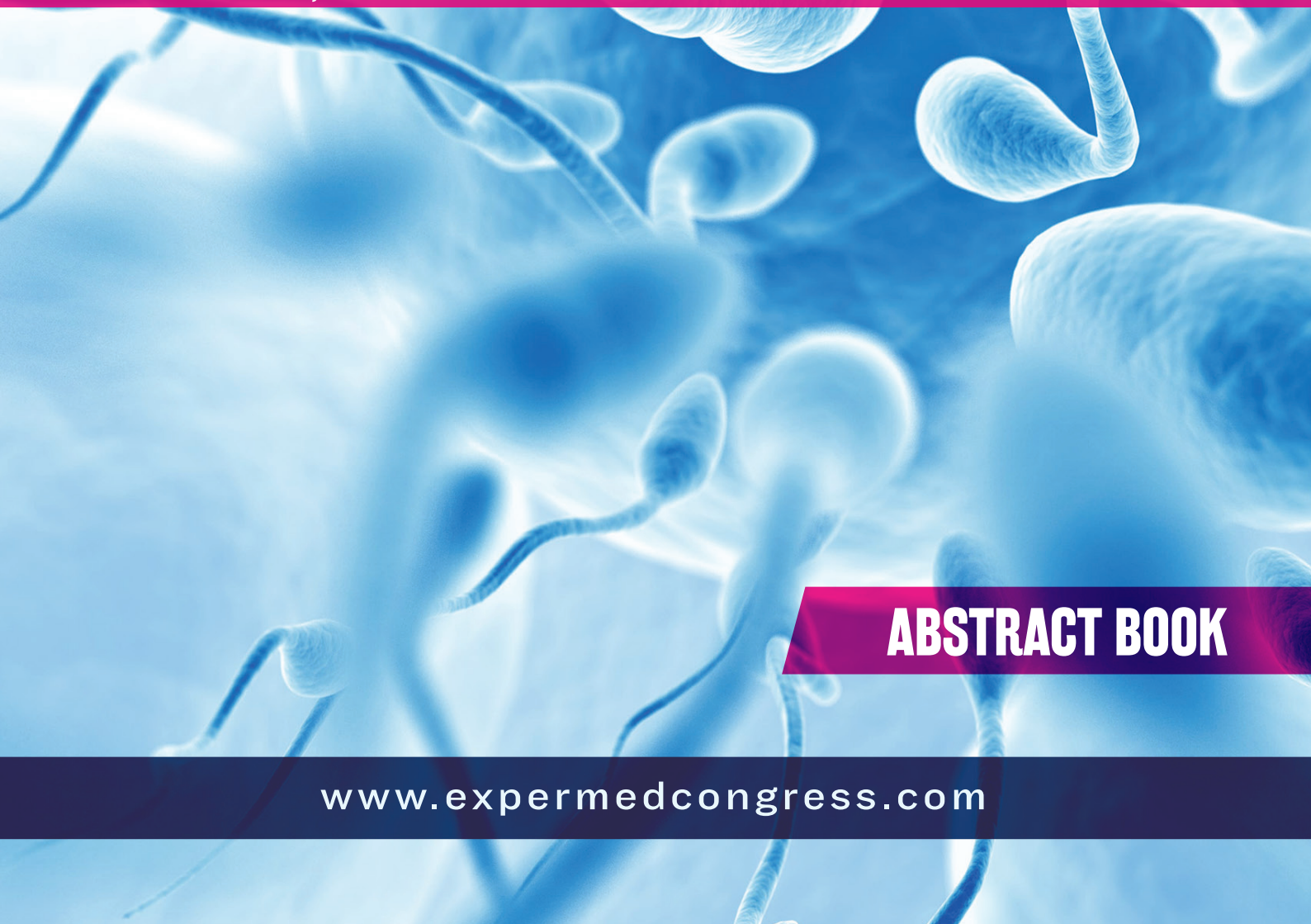
III. INTERNATIONAL

EXPERMED CONGRESS

EXPERT MEETING IN PERSONALIZED REPRODUCTIVE MEDICINE

11-14 APRIL, 2019 | LIMAK CYPRUS DELUXE HOTEL

BAFRA, TURKISH REPUBLIC OF NORTHERN CYPRUS



ABSTRACT BOOK

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SPEAKER ABSTRACTS

EXPERT MEETING IN PERSONALIZED REPRODUCTIVE MEDICINE

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Carlos SIMON

Personalized Reproductive Medicine to maximize reproductive success

Carlos Simón, M.D., Ph.D.
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Adjunct Clinical Professor Ob/Gyn. Baylor College, Houston, TX
Head of the Scientific Board of Igenomix

The most fundamental reason why IVF treatments are not successful is because transfer of good quality embryos or even euploid embryos into the endometrial cavity does not lead to pregnancy. Globally, current live birth rates range between 25% and 30% per initiated cycle (1), and has not improved in the last decade. ART continue to evolve, and the future of medically-assisted procreation cannot be conceived without personalization of the two main players of the implantation process namely the embryo and the maternal endometrium using the existing state of the art scientific advances.

The human endometrium is a hormonally regulated organ that is the anatomic pre-requisite to initiate and control human pregnancy while is a sentinel for protecting the upper female reproductive from infections by a variety of microbes. It undergoes cyclic cellular proliferation, differentiation, lymphangiogenesis, immune cell trafficking, and, in the absence of pregnancy, tissue desquamation and hemostasis, followed by regeneration.

In this presentation, we will discuss the contribution of the endometrial factor to the reproductive process. Our initial understanding of the endometrial microbiome; the endometrial cavity has been classically considered to be a sterile organ, but reports challenging this dogma support the existence of an endometrial microbiota composed of different microorganisms (specifically Lactobacillus spp., Mycoplasma hominis, Gardnerella vaginalis, and Enterobacter spp.) that affect the reproductive outcome (2). The embryo-endometrial dialogue; we have identified a novel cell-to-cell communication mechanism that involves the delivery of endometrial miRNAs from the maternal endometrium to the pre-implantation embryo. Exosome-associated and free hsa-miR-30d was taken up by the embryo from the EF. Trophectoderm cells are able to take up maternal miRNAs; these miRNAs are proposed to be incorporated into the RISC complex to exert gene regulation under physiological conditions, thereby resulting in the observed modifications to the transcriptome and embryo adhesion (3). The personalized molecular diagnosis of the endometrial window of implantation and its clinical translation in patients with recurrent implantation failure (4). Finally, we provide evidence of a decidualization

defect in the endometrium of women with severe preeclampsia (PE) that was detected at the time of delivery and persisted years after theaffected pregnancy. We went on to link this defect to impaired cytotrophoblast invasion. The transcriptional signature of the defect could enable its detection before (or after) conception, which would aid the development of therapies focused on improving decidualization and perhaps preventing severe PE (5).

Precision medicine supported by contrasted basic research together with clinical translation in an evidence-based medicine approach should guide the improvement of our field

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EXPERT MEETING IN PERSONALIZED REPRODUCTIVE MEDICINE

Carmen RUBIO

Non-invasive embryo selection: Is this the future or an ilusion?

Carmen Rubio
Research Director at Igenomix for non-invasive PGT-A.
PhD in Genetics and Embryology.

The most common genetic abnormality identified in human embryos is aneuploidy. This abnormality is particularly common among embryos produced by in vitro fertilization (IVF), more than half are aneuploid. For this reason, identifying the euploid embryos with the highest potential to implant and establish ongoing pregnancy is crucial.

In the last years there have been different attempts to overcome trophectoderm biopsy to diagnose the chromosomal content of the embryos. Some groups started with the analysis of blastocoel fluid obtained by aspiration with a thin micropipette as a less invasive approach than TE biopsy. Later on, some groups proposed a "true" non-invasive approach consisting in the study of the spent culture media to analyse the embryonic cell free DNA (cfDNA) released by the embryo during the latest stages of preimplantation development. After the first publications, several studies have compared the results of PGT-A in TE biopsies with the results of the spent culture media, to establish the concordance rates among both approaches

Furthermore, in another study the results of the combination of blastocoel fluid and spent culture medium was compared with TE and with the whole blastocysts in study, with higher concordance rates between media and whole blastocyst embryo than between the TE and the whole blastocyst, pointing out that blastocoel fluid and spent culture medium could be more representative of the 'true' chromosomal content of the embryos than trophectoderm.

In a recent study, we have assessed the informativity and concordance rates of TE biopsies with the analysis of the cfDNA in the culture media, with high informativity rates when the blastocyst media were collected after 48 hours in culture and with 84.0% concordance rate with the TE biopsy and this results will be presented in the lecture as well as new ongoing studies in this field.

EXPERT MEETING IN PERSONALIZED REPRODUCTIVE MEDICINE

Glenn SCHATTMAN

Associate Professor of Reproductive Medicine at the Weill Medical College of Cornell University

Glenn Schattman

Recent advances in cryopreservation technology have allowed for better survival and overall success from a single cycle of ovarian stimulation. Rather than using excess embryos to augment the probability of pregnancy in an ART cycle, the pendulum has swung the other way and embryos are being cryopreserved primarily without ever proceeding with a fresh transfer. There are many potential reasons for this however, this presentation will review the reasoning for potentially not performing fresh embryo transfers and the risks and potential benefits of cryopreservation with transfer of an embryo in a more physiologic, unstimulated cycle.

EXPERT MEETING IN PERSONALIZED REPRODUCTIVE MEDICINE

Anja PINBORG

Should we use elective freezing strategies - Does and donts

Anja Pinborg

Vitrification and blastocyst transfer have considerably improved success rates after frozen embryo transfer (FET) with ongoing pregnancy rates in frozen cycles approaching those seen in fresh treatment cycles. Furthermore, the risk of ovarian hyperstimulation syndrome (OHSS) is essentially eliminated in FET cycles, and FET may be beneficial to the endometrial and foetal development because a hormonal environment mirroring the natural cycle is enabled. Moreover, elective freezing strategies facilitate single embryo transfer lowering multiple birth rates. However, should the freeze-all strategy be implemented as standard care. This lecture will summarize the current evidence for success rates in freeze-all versus fresh embryo transfer strategies and make an overview of perinatal outcomes after FET. Further patient's perceptions of freeze-all strategies and indications for freeze-all will be discussed.

EXPERT MEETING IN PERSONALIZED REPRODUCTIVE MEDICINE

Christian BECKER

Molecular diagnostics in endometriosis: An update

Christian Becker
Associate Professor
University of Oxford

Endometriosis is a common chronic inflammatory condition affecting millions of women worldwide. It is associated with pelvic and abdominal pain, painful sexual intercourse and infertility. There still exists a long delay between the onset of the disease and its diagnosis. Laparoscopy remains the diagnostic gold standard, but it is invasive and as such associated with morbidity and mortality.

The presentation will focus on current approaches towards the discovery of non-invasive methods to diagnose and rule out endometriosis in symptomatic women. Recent developments will be critically appraised and comparisons with other conditions will be made.

EXPERT MEETING IN PERSONALIZED REPRODUCTIVE MEDICINE

Bülent URMAN

REPRODUCTIVE SURGERY IN 2019-WHAT HAS CHANGED IN THE LAST 40 YEARS

Dr. Bulent Urman
Koc University School of Medicine
American Hospital, Istanbul

Reproductive surgery has not changed, it has evolved. Reproductive surgery is not mainstream surgery performed by mainstream surgeons. It requires special expertise and sufficient knowledge regarding the anatomy and function of the female genital organs as they pertain to fertility. Reproductive surgery is often performed for alleviation of infertility, however, more recently it is being increasingly utilized for patients with fibroids, endometriosis, and congenital or acquired uterine abnormalities who are not interested in becoming pregnant immediately but also who wish to preserve their reproductive potential.

Population based studies show that tubal reconstructive surgery such as adhesiolysis, fimbrioplasty, neosalpingostomy, reversal of sterilization and tubocornual anastomosis are being performed much less compared to 20 years ago. This can mainly be attributed to the immediate success that is provided by assisted reproductive technologies. Lack of properly designed prospective, and randomized studies have greatly hampered the embracement of tubal reconstructive surgery by physicians who are more than ever hungry for evidence-based data. Tubal reconstructive surgery is difficult and requires properly trained surgeons in microsurgical techniques. With the advent of IVF, demand for microsurgical training has reached an all-time low, thus, making it difficult if not impossible for the patient to find a surgeon who is trained do perform these operations.

Fertility outcome of tubal reconstructive surgery in the presence mild pelvic adhesive disease or surgical tubal interruption for sterilization is very successful with different authors reporting delivery rates in the range of 60-80% over three years. Tubal surgery, under these circumstances offers the couple a chance at spontaneous and at times multiple conceptions. This may be preferable in young couples where the male partner is fertile and also circumvents some of the adverse side effects of IVF such as ovarian hyperstimulation, increased risk of birth defects and epigenetic problems. However, tubal reconstructive surgery performed for severe

pelvic adhesions, bipolar tubal obstruction, and hydrosalpinges with poor prognostic factors carries a dismal prognosis and should at best be avoided. Instead, removal of severely affected tube/s may be beneficial to the patient in terms of IVF outcomes. Proximal tubal occlusion can be dealt with microsurgical tubal anastomosis, however, this also is a difficult procedure to master and many if not all infertility specialists refer these patients to IVF.

Endometrosis surgery has taken great strides in the last 20 years. Not only we have learnt that judicious application of endometrioma surgery may be detrimental to ovarian reserve, we also witnessed the advances in ultrasound imaging and surgery for deep endometriosis and adenomyosis. Once a surgery only reserved for the few elites reproductive surgery has become a widely practiced procedure in the last 20 years.

Surgery for myomas are more often performed by hysteroscopy and laparoscopy and laparotomy is now reserved for patients with very many fibroids not amenable to endoscopic resection. Intraoperative ultrasound also facilitates the removal of relatively small fibroids embedded deep into the myometrium. Mechanic morcellators and application of bipolar energy have enabled the resection of larger submucous fibroids with lesser complications.

Reproductive surgery should definitely be viewed as a complementary not competitive to IVF. Many patients benefit from an indicated and properly performed reproductive surgery by experienced reproductive surgeons. One thing that is becoming more evident is, like oncological surgery, reproductive surgery should only be performed by trained experts in this field. Reproductive capacity of a women should not be viewed as a function that can be sacrificed due to the presence of an IVF back-up option. This would be the same as being content with suboptimal surgery in the cancer patients due to the presence of chemo-radiotherapy.

EXPERT MEETING IN PERSONALIZED REPRODUCTIVE MEDICINE

Istvan ARGAY

Hysteroscopic vs. laparoscopic management of the "Niche": When to treat isthmocele?

Istvan Argay, MD
University of Debrecen Department of Obstetrics and Gynecology

Cesarean delivery is one of the most common surgical procedures in women, with rates of 30% or more in the United States. As a result, the rate is rising for cesarean scar defect—the presence of a "niche" at the site of cesarean delivery scar—with the reported prevalence between 24% and 70% in a random population of women with at least one cesarean delivery. After the etiology we discuss the possibilities of the repair and its necessity. When to choose Laparoscopy or hysteroscopic , and their outcomes.

EXPERT MEETING IN PERSONALIZED REPRODUCTIVE MEDICINE

Stephan GORDTS

Fertility optimization by surgery: What to remove, when to remove?

Stephan Gordts

The introduction of in-vitro fertilization within reproductive medicine has prompted questions to be asked about the relevance of reproductive surgery.

More than a competing discipline, reproductive surgery is complementary to the tech-niques of IVF. As a complementary discipline reproductive surgery covers the field not only of tubal and ovarian pathology but also this of correction of uterine alterations.

Uterine pathology

Underestimated for several years, the last decades more interest is paid to the role of the uterus as a major key player in conception and pregnancy outcome.

The prevalence of uterine congenital anomalies varies from 0.06% in the general popu-lation to 13% in women with history of recurrent spontaneous miscarriages. Hyster-oscopic metroplasty dramatically improves the pregnancy outcome, decreasing the abortion rate and increasing term deliveries; however the place of metroplasty in infertili-ty remains controversial. Confusion is created because of a lack of standardization of diagnostic modalities and measurements. The use of a new and simple to use classifica-tion system (ESHRE-ESGE) and standardization of diagnostic procedures should finally clarify the importance of these pathologies and the necessity of surgical correction.

Concerning myoma, the meta-analysis of Pritts et al. showed a negative impact on pregnancy rates and an improvement after myomectomy in case of submucous myoma. No negative impact could be demonstrated in case of subserous myoma. Most confus-ing are the data on intramural myoma. Data show that a possible negative impact of my-oma on implantation and pregnancy rate is becoming more important as they are closer located to the uterine cavity and junctional zone with a possible important impact through disturbed uterine peristalsis.

Tubal pathology

The beneficial effect of salpingectomy on IVF outcome in cases of thick- walled hy-drosalpinges or ultrasonographically visible hydrosalpinges has been reported by sever-al studies. A Cochrane review showed that salpingectomy pre-IVF resulted in 1.75-and 2.13-fold higher odds of pregnancy and live birth, respectively. When salpingoscopy can exclude the presence of intra-tubal mucosal adhesions a subgroup with more than 50% intra uterine pregnancy rates and less than 5% ectopic rate after reconstructive surgery can be identified. Functional restoring surgery is indicated in good prognosis patients with thin walled hydrosalpinges with minimal or no mucosal adhesions and ab-sence of severe tubo-ovarian adhesions.

Ovarian endometrioma

Although number of oocytes and fertilization rates tended to be decreased in endome-triosis patients, pregnancy rates after IVF have been reported equal to IVF results in pa-tients without endometriosis. A possible oocyte factor can play a major role in this pro-cess as oocytes of endometriosis positive donors result in lower pregnancy rates com-pared to oocytes from controls without endometriosis. Surgery for ovarian endometrio-ma caries the intrinsic risk of damaging the ovarian reserve and should therefore be car-ried out with the highest precision and expertise to keep the ovarian damage to a mini-mum. Treatment should be individualized taken into account the size of the endometri-otic cyst, unilateral or bilateral localization, recurrence, age, pain and the wish to con-ceive.

Conclusion

The role of surgery in reproduction is double: competing and complementary. Except of creating the possibility for spontaneous conceptions, It offers an added complementary value in ameliorating pregnancy rates and live birth rates in patients referred to IVF pro-grams

As it is required for ART procedures, continuous training and accreditation programs are necessary for reproductive surgery as the required expertise is even now more important as earlier.

EXPERT MEETING IN PERSONALIZED REPRODUCTIVE MEDICINE

Joseph NASSIF

Is there a place for surgery in patients with endometriosis and repeated IVF failures?

Joseph Nassif

The use of Assisted reproductive techniques in endometriosis has proven to be efficient and successful. However, some endometriosis patient do not achieve pregnancy after many trials. In this lecture, we will discuss the evidence and updates on the use of surgery to enhance the fertility in endometriosis patients with repeated IVF failures.

EXPERT MEETING IN PERSONALIZED REPRODUCTIVE MEDICINE

Carlo ALVIGGI

Individualization vs standardization

Carlo Alviggi
Department of Neuroscience, Reproductive Science and Odontostomatology
Università "Federico II", Naples, Italy

The main goal in IVF is to offer strategies tailored to women individual characteristics. The aim is to maximize the change of pregnancy and in the same time to minimize side effects and cycle cancellations. A number of predictive variables for ovarian response have been identified, including hormonal, functional and genetic biomarkers.

Among the hormonal biomarkers, anti-Müllerian hormone (AMH) has been shown to have the highest predictive value. AMH is produced by the granulosa cells of early developing follicles and plays a crucial role in regulating the progression of smaller pre-antral follicles. It also modulates the activity of follicle-stimulating hormone (FSH) in antral follicles during the FSH-dependent growth stage. Antral follicle count (AFC) is a well known functional biomarker that is used to predict ovarian response to stimulation and is also an important factor in determining the optimal starting FSH dose for ART.

The importance of such markers in clinical practice is confirmed by the fact that ovarian reserve markers were used to titrate gonadotropin dosage during ovarian stimulation. Despite

individualization offers several advantages in terms of compliance and in women at risk of hyper-response some concerns have been raised for the management of low prognosis women with low ovarian reserve. Furthermore, the superiority of individualized versus standardized treatments in terms of live births or ongoing pregnancy rate is still a matter of debate

EXPERT MEETING IN PERSONALIZED REPRODUCTIVE MEDICINE

Danilo CIMADOMO

Effect of IVF laboratory on euploidy outcome

Dr. Danilo Cimadomo, MSc, PhD

A paper published in 2017 claimed that significant difference may exist in the euploidy outcomes among different IVF centers. In this lecture, we will go through all the lab procedures required to conduct PGT, and their putative impact on the euploidy outcomes, aiming at finding an explanation for those data.

Controlled ovarian stimulation (COS): several papers have been published across the years, yet an effect of COS dosage or protocol on the euploidy rates is not sustainable. An evidence confirmed also i) in patients undergoing two consecutive cycles: one unstimulated and one stimulated, ii) in poor responder patients, as well as iii) in patients undergoing double ovarian stimulation in the same ovarian cycle and collecting oo-cytes from both the follicular and the luteal phase.

ICSI and male factor infertility: ICSI seems not to affect the euploidy rates when compared to IVF. Similarly, severe male factor has an impact on oocyte fertilization and developmental competence to blastocyst, but not on the chromosomal constitution of the blastocyst(s) obtained. Moreover, artificial oocyte activation might be investigated as a clinical option, when required, as well as the enhanced chromosomal analysis of abnormally fertilized oocytes (e.g. 1PN) to rescue them for the clinical use.

Embryo culture: Embryos undergoing extended embryo culture up to day7 (about 5%) might still be eu-ploid and implant. Indeed, a correlation mainly exist between euploidy and blastocyst morphological qual-ity. Yet, also poor quality blastocysts (<BB) are euploid in about 25% of the cases and, even if showing a sig-nificantly lower chance of implantation, they do result in healthy live births. No paper has ever reported an effect of sequential or continuous embryo culture on the euploidy outcomes. Undisturbed embryo culture does not seem to involve better clinical outcomes when compared to standard incubation. Moreover, morphodynamic parameters of embryo culture assessed in time-lapse showed contrasting results for their putative prediction of euploidy to date. Nevertheless, this tool might be useful to highlight unconventional patterns of cell division implicitly associated with high risks for chromosomal imbalances.

Biopsy: The approach chosen for embryo biopsy clearly has an effect on the euploidy outcomes mainly be-cause of four reasons: single cell analysis issues on polar bodies and blastomeres, lower informativity of po-lar bodies and blastomeres as a consequence of technical/biological issues (e.g. exclusion of paternal meiot-ic errors, mosaicism, etcetera), impact of blastomere biopsy on embryo implantation potential, and

repro-ducibility of the biopsy outcomes across experienced and unexperienced operators. Some papers reported high reproducibility (no significant difference) of the molecular outcomes across several operators from the same IVF unit and good reproducibility across different units. Yet, the IVF center, mainly due to diverse training and internal key performance indicators, is a variable that might have a slight influence on the technical outcomes after PGT.

Mosaicism: Euploid/aneuploid mosaicism represents an issue for the diagnosis. However, at the blastocyst stage, this risk accounts for only 5% of the embryos. Therefore, the real issue is represented by the attempt of diagnosing mosaicism based on a single trophectoderm biopsy (about 20% of euploid/aneuploid mosai-cism reported), since it exposes to high risk of false diagnoses. Non-selection studies a required to shed light on the real clinical value of the practice of reporting mosaicism based on a single biopsy.

Vitrification: Oocyte vitrification has no effect on chromosomal constitution evaluated at the blastocyst stage. These are the data of a randomized controlled trial on sibling oocytes and of an observational study of the effect of long-term storage.

A thorough inspection of the literature in this topic outlined that the main risk for slightly different eu-ploidy outcomes across several IVF units is mainly imputable to the efficiency of the operators in conduct-ing embryo biopsy. Proper training, a constant monitoring of each operator's performance, as well as a pro-active communication between embryologists and molecular biologists/geneticists are crucial to improve the equality of a PGT program.

Total genetic risk of an individual encompasses not only inheritable conditions, but also genetic condi-tions whose incidence is not purely hereditary (i.e., oocyte chromosomal aneuploidy, de novo muta-tions). The most common reason for chromosomal aneuploidy is the accumulation of errors in the mei-otic machinery, which results in significantly inefficient germinal cell maturation process during meiosis. There is solid evidence that the incidence of aneuploidy in embryos generated by women of advanced maternal age is significantly higher than in younger patients. This defective process impacts the ability of females of advanced maternal age to naturally conceive a healthy baby. The only effective strategies to assess the presence of non-hereditary chromosomal abnormalities in embryos and fetuses remain preim-plantation and prenatal genetic testing. Genetic disorders caused by de novo mutations (DNMs) in auto-somal dominant genes cannot be detected by preconcaeption carrier screening, as these mutations are not present in the parental DNA routinely isolated from blood samples. Germline DNMs are now well known to play an important role in severe early-onset diseases, which for the most part arise sporadically because of their impact on fitness; they are mostly of paternal origin and their number increases with advanced paternal age. However, the overall genetic disease risk provided by the 700 genes associated so far to the DNMs remain modest, explaining around 0.1% of the neonatal risk for severe conditions.

If expertly reasoned and properly performed, both ECS and PGT are effective approaches to mitigate the reproductive genetic risk of couples trying to conceive, reducing the uptake of invasive PND and anxiety in prospective parents.

EXPERT MEETING IN PERSONALIZED REPRODUCTIVE MEDICINE

Antonio CAPALBO

How important is to detect the genetic risk of a couple trying to conceive?

Dr Antonio Capalbo, PhD
Laboratory Director, Igenomix Italy
Research Project Director at Igenomix, Spain.

It is estimated that approximately 30 in 10,000 children suffer from one of the 1,300 recessively inherited disorders (autosomal and X-linked). This means that 1-2% of couples are at high risk of having an affected child. In past years, standard practice involved testing carrier status of population at-risk only: adult individuals with family history of a particular recessive disease; partners and relatives of identified carriers; specific ethnic groups at increased risk (i.e. Ashkenazi), focusing on a selection of most frequent disorders. This way, however, only a minority of carrier couples are identified, as the majority of affected children are born to couples with unknown family history, and only a fraction of high-risk families re-quest carrier testing. Recent advances in Next Generation Sequencing (NGS) have led to the development of Expanded Carrier Screening (ECS) platforms, which allow universal testing application for populations with different ethnic background, parallel testing of a greater number of disorders, and significantly re-duced costs.

Studies on the application of ECS testing revealed that the cumulative risk for healthy individuals to con-ceive a child with a recessive condition is comparable to the risk for Down's syndrome in the general population. Through preconception ECS, carrier couples at risk of transmitting a severe genetic condi-tion to their offspring can be identified, thus making them aware of their reproductive risk and allowing them to make an informed decision about their reproductive options (i.e., using preimplantation diag-nosis, donor gametes, or adoption).

EXPERT MEETING IN PERSONALIZED REPRODUCTIVE MEDICINE

Carlo ALVIGGI

Number of oocytes retrieved

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Conceivement of a healthy child is widely considered the most important target in IVF. Several analysis including thousands of IVF births demonstrate that the number of oocytes retrieved is the most accurate predictor of live births. In detail, the higher is the number of oocytes collected the greater are the likelihood of childbearing. In fresh cycles, emerges that the appropriate number of oocytes to maximize live births stands between 10 and 15. On the other hand, when cumulative births coming from both fresh and frozen/thawed cycles was considered, even the collection of more than 15 oocytes could still have some benefit. Thus, every strategies that allow the retrieval of as much as possible oocytes during stimulation should be pursued, especially when cryopreservation is planned. Nonetheless, the number of oocytes collected should be linked to the natural reduction of euploidy rate registered in advanced age patients. Indeed, the number of oocytes we need in advanced age women to obtain at least one euploid blastocyst (> 10 oocytes) is greater than in younger women (≈ 6 oocytes). In order to estimate the adequate number we need per cycle to obtain at least one euploid embryo, Poseidon group are developing a novel calculator which predict the precise number of oocytes we need at the end of ovarian stimulation through several qualitative and quantitative predictors.

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Luciano NARDO

Professor Luciano Nardo, MD MRCOG
Clinical Director Reproductive Heath Group
Consultant Gynaecologist

EXPERT MEETING IN PERSONALIZED REPRODUCTIVE MEDICINE

Diego EZCURRA

How will innovation transform our current IVF practice: A glimpse from the near future

Diego Ezcurra

The embryology lab is the place where all the formulas and technologies are being applied to make the best, of the raw materials we receive from the patients, to maximize the production of embryos with high implantation potential that can achieve a pregnancy and a live birth. Embryologist are experts and like chefs everyone has is own formulas and ways of doing things. Lab processes involve hundreds of variables that we try to control to achieve levels of excellence and maximize outcomes but there is a huge variabil-ity in processes and assessments between labs and within same lab as well as a huge component of sub-jectivity that needs to be replaced by objectivity at every level. During my presentation I would like to go over a futuristic vision of breakthrough innovation in the embryology lab leading to standardization, automation and levels of excellence in lab processes to increase clinical and operational efficiencies.

EXPERT MEETING IN PERSONALIZED REPRODUCTIVE MEDICINE

Nathan TREFF

ASSESSMENT OF GAMETE & EMBRYO QUALTY: CAN mtDNA-BASED TESTS BE THE ANSWER?

Nathan R. Treff, PhD, HCLD

The use of mtDNA quantitation has not been validated for clinical use. Conflicting data on clinical pre-dictive value, and the lack of a randomized clinical trial, makes its routine use in clinical care unwarrant-ed. In fact, all studies to date indicate that mtDNA quantity may simply measure the developmental stage of the embryo, which is already accomplished with morphological assessment. Furthermore, these limitations have now been acknowledged by a leading commercial provider and discontinued as a clini-cal service. Together, these observations make it clear that mtDNA-based tests are not the answer to im-proved assessment of gamete and embryo quality.

EXPERT MEETING IN PERSONALIZED REPRODUCTIVE MEDICINE

Xavier SANTAMARIA

ASHERMAN'S SYNDROME PROJECT

Xavier Santamaria MD, PhD
Director of the Asherman Project at Igenomix

Asherman's syndrome (AS) is an uncommon gynaecological disorder caused by the destruction of the endometrium due to repeated or aggressive curettages and/or endometritis. As a result, there is a loss of functional endometrium in many areas and the uterine cavity is obliterated by intrauterine adhesions, leading to amenorrhea, hypomenorrhea, infertility, recurrent pregnancy loss and/or ab-normal placentation, including placenta previa and accreta.

The prevalence of AS is estimated to be 4.4/10000 person and CD133+ has recently obtained Or-phan Drug Designation (ODD) in Europe (EMA) and the US (FDA).

Our group managed performed a prospective, experimental, non-controlled investigator-driven pilot study that showed thad CD133+ cells could be effective and safe for the treatment of refractory AS and/or EA in women of fertile age with a wish to conceive.

This proof of concept study included 16 patients (30–45 years) with refractory AS (n = 11) or EA (n = 5). After the initial hysteroscopic diagnosis, BMDSC mobilization was performed by G-CSF injection, after which CD133+ cells were isolated through peripheral blood aphaeresis to obtain a mean of 124.39 million cells (range 42–236), which were immediately delivered into the spiral arte-rioles by catheterisation (Figure 1). Results demonstrated that in the first 3 months, autologous cell therapy with CD133+ BMDSCs in conjunction with hormonal replacement therapy (HRT) in-creased the volume and duration of menses as well as the thickness and angiogenesis processes of the endometrium while decreasing the intrauterine adhesion scores (Santamaria et al., 2016) in a statistical significant manner. Regarding reproductive outcomes 7 pregnancies out of 14 transfers where achieved (PR=50%). Additionally, 3 patients become spontaneously pregnant 2, 4 and 22 months after cell therapy. From the 8 pregancies, 5 Healthy New Born were delivered from 4 pa-tients and there was 1 Ectopic Pregnancy, 3 Biochemical Miscarriages and 2 Clinical Miscarriages (one at 9 weeks due to abnormal embryo and an other with PRM at 17 weeks after amniocentesis).

Additionally, we analyzed cell engraftment and localization by Prussian blue staining, endometrial proliferation by Ki67 staining and quantitative reverse transcriptase–polymerase chain reaction (qRT-PCR) of marker genes in a mouse model of AS. These results demonstrated that human CD133 BMDSCs induced indirect proliferation of the neighbouring endometrial cells in the dama-ged endometrium mainly at the epithelial compartment. The endometrial cell proliferation observed appears more related to the secretion of soluble factors such as bone morphogenetic protein 6 (Bmp6), platelet-derived growth factor β (Pdgfβ), thrombospondin 1 (Thbs1) acting by a paracrine fashion than a direct proliferation of the transplanted cells. We are currently performing GLP (Good Laboratory Practice) studies in rats to better determine biodistribution, long-term sa-fety and mechanisms of action.

EXPERT MEETING IN PERSONALIZED REPRODUCTIVE MEDICINE

Hamish WALLECE

Fertility preservation: a global view

WH Wallace
Professor W. Hamish Wallace
Professor of Paediatric Oncology,
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Royal Hospital for Sick Children,
Edinburgh EH9 1LF

Some cancer treatments may affect fertility for both males and females, and loss of fertility is a major concern for survivors. If the planned treatment puts the patient at high risk of infertility, fertility preservation options should be considered and discussed with the patient before treatment commences.

For girls and young women without a partner ovarian tissue cryopreservation remains experimental although there are now around 130 reported live births following cryopreservation of ovarian tissue and reimplantation after thawing at a later date.

For young men who have completed puberty who are able to masturbate and ejaculate, there is the well-established option of semen cryopreservation. Current recommendations are that all adult men and teenage boys who are due to receive chemo or radiotherapy that might impair their fertility should be offered the option to cryopreserve their semen. The decision in younger patients depends on a clinical assessment of pubertal stage and emotional maturity. However, for pre-pubertal patients and pubertal patients that are not able to produce a semen sample, approaches for fertility preservation remain experimental and are only available in a limited number of centres worldwide usually under the auspices of a clinical trial.

For young patients who do not have the capacity to give informed consent the first challenge for the clinician is to do no harm (Primum Non Nocere) and be aware that experimental interventions in children can only be ethical if they can be considered to be therapeutic and in the best interests of the child.

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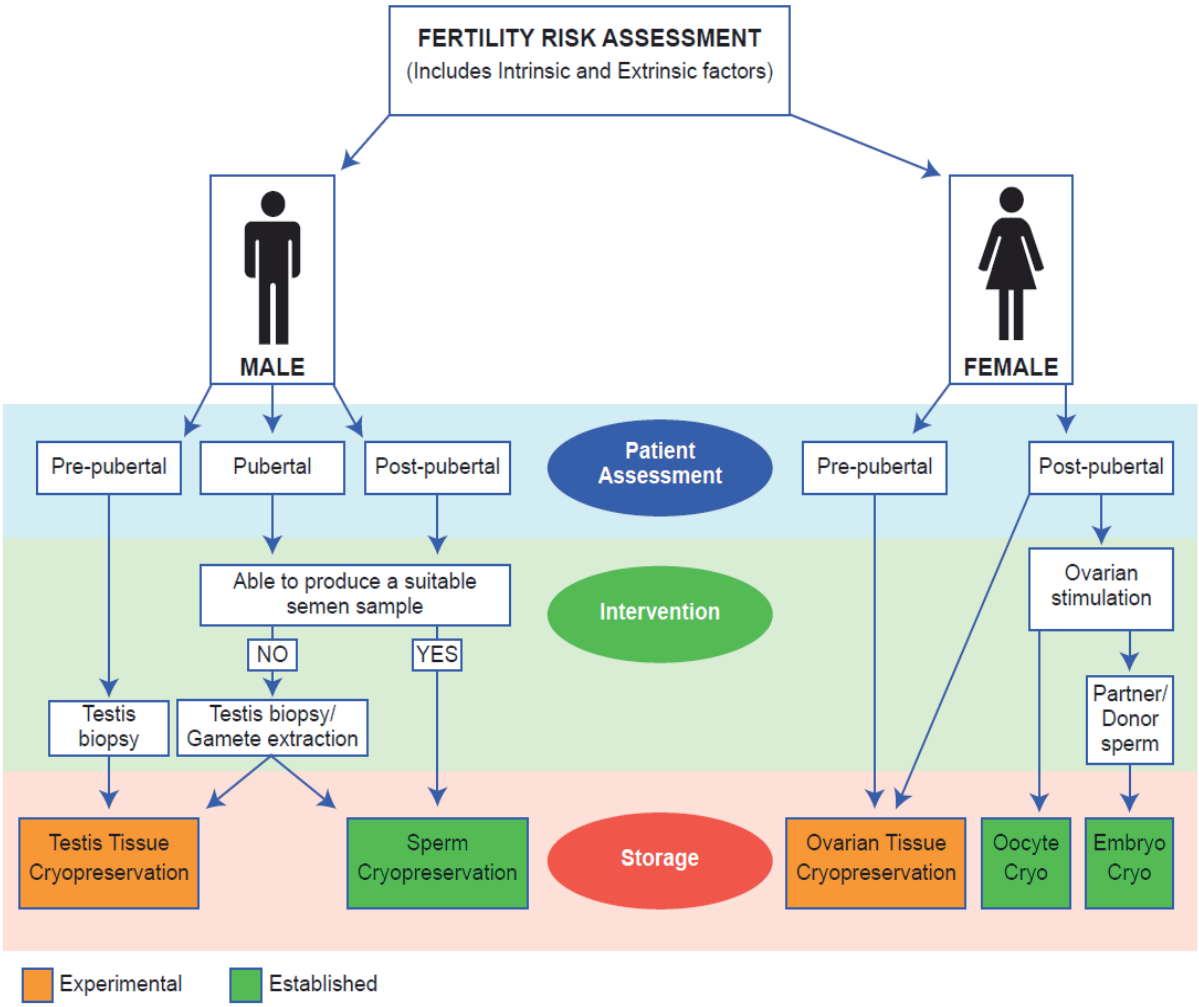

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EXPERT MEETING IN PERSONALIZED REPRODUCTIVE MEDICINE

Richard ANDERSON

AMH in the modern era of assisted reproduction and fertility preservation

Professor Richard Anderson
University of Edinburgh

Since its origins in developmental biology in the 1950s, AMH has become a mainstay of modern repro-ductive endocrinology. In females, the demonstration that it is produced by granulosa cells of growing follicles, yet with reduced expression in larger and preovulatory follicles has led to its most established role as a marker of what can be called the 'functional' ovarian reserve, as a predictor of response to ovari-an stimulation. Assay development has moved from manual ELISA plates to now automated assays, thus it takes it place alongside other routine measurements in the modern biochemistry lab. Other applica-tions remain less established, and although it has clear value in the assessment of the ovary in PCOS, it has yet to become part of the diagnostic criteria. In oncology, AMH levels before chemotherapy predict long-term ovarian function, and clearly discriminate between the degree of gonadotoxicity of different treat-ment regimens. That AMH is produced by the ovary in childhood allows assessment of ovarian function before puberty for the first time, and at the other end of the reproductive lifespan it is becoming estab-lished as part of the diagnosis of the menopause.

EXPERT MEETING IN PERSONALIZED REPRODUCTIVE MEDICINE

Evelyn TELFER

Growing Human Eggs In Vitro
Professor Evelyn E Telfer
Institute of Cell Biology, The University of Edinburgh

Removal and storage of ovarian cortical tissue is currently offered to young female cancer patients undergoing potentially sterilizing chemotherapy and/or radiotherapy. For patients at high risk of re-introduction of malignancy through auto-transplantation, the ultimate aim is to achieve complete oocyte development from this tissue in vitro. The ability to develop human oocytes from the earliest follicular stages through to maturation and fertilisation in vitro would revolutionise fertility preservation practice. This has been achieved in mouse where in vitro grown (IVG) oocytes from primordial follicles have resulted in the production of live offspring.

For many years we have been developing systems that support growth and development of oocytes from human ovarian cortex. We have now developed a multi-step culture system that supports the development of some human oocytes from immature follicles through to meiotic maturation demonstrated by the formation of polar bodies and a Metaphase II spindle. This system provides a model system to study the regulation of human oocyte/follicle development from activation to maturation.

This presentation will give an update on the feasibility of recapitulating many of the steps involved in human oogenesis under in vitro conditions using tissue from a range of patient groups and consider the steps that will be required to determine whether IVG could be used in a clinical setting.

EXPERT MEETING IN PERSONALIZED REPRODUCTIVE MEDICINE

Ying CHEONG

Recurrent imp failure: What does it mean? Why does it happen?

Ying Cheong
Professor of Reproductive Medicine, Medical Director, Complete Fertility

Reproductive science is still in its infancy; for every couple that enjoys the success of assisted conception as a means of conception, many more face the distress of failed treatments. In medicine, where treatment success is limited and world-leading scientists and medical specialists are grappling with lessons learnt from failures related to the medical science, clinicians face the conundrum of what treatment to adopt next for those who experience repeated failed treatments. Many advocate the use of adjuvant and com-plementary treatments for patients with recurrent implantation failure (RIF), although the vast majority of these therapies are based on biological plausibility rather than current evidence of efficacy. It is crucial to bear in mind that before one adopts non-evidence-based treatment strategies, factors associated with the success and failure of in vitro fertilisation treatments should be reviewed and addressed. These in-clude optimising modifiable lifestyle factors (such as diet, exercise, smoking and alcohol consumption); addressing contributory surgical factors (such as uterine/tubal/ ovarian pathologies and anomalies); and assessing the level of difficulties in embryo transfer of previous cycles, as well as re-evaluating the pa-tient's history and investigations. Success or failure, undergoing IVF is often a long and hard journey for many. So much can be achieved if the clinicians place emphasis on managing the patient's expectations from the outset. In the practice of reproductive medicine, to be honest with our patients regarding the limits of reproductive science, and at times to adopt a supportive doctoring role rather than instituting non-evidence-based interventions can be the right approach. This presentation aims to provide a critical-ly appraised evidence-based overview of current management strategies for the couples with RIF.

EXPERT MEETING IN PERSONALIZED REPRODUCTIVE MEDICINE

Joyce HARPER

Is PGT-A the answer to recurrent implantation failure?

Professor Joyce Harper
Institute for Women's Health, University College London

Since the structure of DNA was deciphered in 1953 by Watson, Crick and Franklin, we have come a long way. By 2001 we had sequenced the first human genome and James Watson had his genome sequenced in 2007 at a cost of $1.5 million. Now it costs under $1000 to sequence a human genome.

Reproductive genetics consists of preconception testing, preimplantation genetic testing and prenatal diagnosis.

New WHO nomenclature has stated that preimplantation genetic diagnosis (PGD) and screening (PGS) should be renamed preimplantation genetic testing for monogenic diseases (PGT-M), preimplantation genetic testing for chromosome structural rearrangements (PGT-SR) and preimplantation genetic testing for aneuploidy (PGT-A).

For PGT, cells can be biopsied from the zygote, cleavage or blastocyst stage, but due to post zygotic mosa-icism, most PGT is now done at the blastocyst stage. Instead of removing cells from the embryo, studies are ongoing to look at blastocoel fluid biopsy and using the spent culture media.

The diagnostic techniques for PGT have changed greatly over the last 30 years, from using fluorescent in situ hybridsation through to next generation sequencing. The evolution has made the tests more efficient and enabled the detection of more genetic information.

PGT-A is an IVF adjunct aimed at increasing the IVF live birth rate. For PGT-A, version 1 used mainly cleav-age stage biopsy and fluorescent in situ hybridization but 11 randomized controlled trials (RCT) found that the procedure did not work. Four underpowered RCTs using PGT version 2 have shown benefits in both good and poor prognosis patients. The recently published ESHRE ESTEEM study shows that there were less transfers and less miscarriages in the PGT-A group but there was no difference in the live birth rate. The STAR trial has not been published yet but shows that overall there is no significant difference in ongoing pregnancy rates, but sub-analysis shows a significant increase in ongoing pregnancy in the 35-40 year old age group. There are no major studies looking at repeated implantation failure and PGT-A. PGT-A accounts for 1% of ART cycles in the UK, but 21% in the USA.

In conclusion, is PGT-A the answer to recurrent implantation failure? At the current time, there is no evidence to show this.

EXPERT MEETING IN PERSONALIZED REPRODUCTIVE MEDICINE

Susan LAIRD

Immune-related ART failures:From theory to reality

Susan M. Laird
Biomolecular Sciences Research Centre, Sheffield Hallam University, UK

The mechanism by which women are able to carry a foetus which expresses paternal and therefore al-logeneic proteins has been the subject of much debate. Despite no clear understanding about these mechanisms it has been suggested that various reproductive disorders such as recurrent miscarriage and ART failure are due to a defect in these mechanisms. Two of the most controversial areas of reproductive immunology are the role of cytokines and uNK cells in reproductive outcome.

Early studies in mice indicated that the balance between TH1 (IL2, TNFα, IFNγ) and TH2 (IL4, IL6, 1IL10) cytokines is important in pregnancy outcome; high levels of TH1 cytokines were associated with preg-nancy failure. Human pregnancy is accompanied by a shift toward a TH2 cytokine profile, probably con-trolled by progesterone, but evidence to suggest that pregnancy loss is due to a failure to make this shift is contradictory. Within the endometrium and decidua there are complex networks of numerous cyto-kines which do not fit the TH1/TH2 theory. For example many studies suggest that LIF is important in embryo implantation both in mice and humans. However, in vitro TNFα stimulates LIF production, con-tradicting the fact that TNFα is a "bad" cytokine.

Uterine natural killer cells (uNKcells) are a population of cells within the endometrium and decidua, whose numbers are highest at the time of embryo implantation, suggesting that they play a role in this important process. Various studies have shown that uNK cell numbers are increased in women with re-productive failure including women with repeated implantation failure (RIF), although there are other studies that show no difference. There is also debate as to whether these high numbers affect pregnancy outcome; some studies show that high numbers are associated with poor pregnancy outcome while oth-ers show no association. The numbers of uNK cells do not reflect their functional state and more needs to be understood about their mechanism of action. uNK cells are able to interact with the trophoblast via Killer Inhibitory Receptors (KIRs) binding to HLA molecules on trophoblast cells and some studies have suggested that some KIR/HLA combinations are incompatible with successful pregnancy.

The lack of overall consensus may be due to differences in study design and methods of measurement. Cytokines are local control mediators; they are produced within tissues and have an effect within the same tissue. Therefore levels in the blood will not reflect those in the endometrium or placenta. Many studies have also compared levels of cytokines at different times in pregnancy or after pregnancy loss in one of the study arms, which would account for differences seen. The NK cell population in the endome-trium and decidua (predominantly CD56 bright CD16-) is very different from that in the blood (predomi-nantly CD56dimCD16+) and again peripheral blood measurements do not reflect the situation in the decidua.

Despite the controversy a number of women are requesting and being offered analysis of NK cells and cytokines and if these are considered "abnormal" could be offered intravenous immunoglobulin, gluco-corticoids, anti-TNFα or intralipid as treatment. However convincing evidence for the effectiveness of these treatments, except perhaps in a few subgroups of women, is not available.

EXPERT MEETING IN PERSONALIZED REPRODUCTIVE MEDICINE

Samuel DES SANTOS RIBEIRO

Clinician's perspective

Samuel Dos Santos Ribeiro
Professor Doctor
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The use of assisted reproductive technology (ART) procedures to treat infertility has developed vastly and increased significantly since its inception in the late 1970s. Despite substantial advancements in the field, this process remains highly inefficient with up to 75% of all the oocytes retrieved being eventually discarded due to immaturity, failed fertilization or poor embryo development.

Follicular development and oocyte competence are two intimately related processes and exogenous ovarian stimulation plays a significant role in both during ART. Most of the studies performed in animals show a deleterious effect of ovarian stimulation on oocyte quality and embryo development throughout different stages. However, the impact of ovarian stimulation on human oocyte and embryo quality re-mains relatively unclear. During this presentation we will examine the evidence regarding specific clini-cal interventions which may maximize oocyte competence.

EXPERT MEETING IN PERSONALIZED REPRODUCTIVE MEDICINE

Marcos MESEGUER

Oocyte Competency; can we interevene?

MARCOS MESEGUER Phd. MStat
Scientific Supervisor and Senior Embryologist
Equipo IVI and IVI Valencia.

The oocyte is the major determinant of embryo developmental competence in women. The oocyte transmits not only the mother's nuclear but also her mitochondrial genome to the embryo, and mitochondrial DNA is known to be especially susceptible to aging. After ovarian stimulation, patients with normal functional ovarian reserves produce a good number of oocytes at retrieval. In addition to quantity, however, the maturity of the oocytes is an important variable for the success of reproductive technologies. In general, nearly 85% of retrieved oocytes are mature and reproductively useful, whereas the remaining 15% are still at the metaphase I or germinal vesicle stage (metaphase I [M1] [4%] and germinal vesicle [11 %], respectively) Immature oocytes are usually unsuitable for reproductive purposes and are generally discarded. This loss of immature oocytes does not usually negatively affect the chances of pregnancy when the ovarian response is optimal; however, in some scenarios divergent stages of oocyte maturation can increasingly be observed, which depends on several factors. For instance, the functional ovarian reserve determines the proportion of mature and immature oocytes available, so that women with a low functional ovarian reserve render a significantly higher percentage of immature oocytes than those with normal ovarian function, An altered ratio of mature-to-immature oocytes can also be determined by the approach used for ovarian stimulation, and by the ovarian response. In this respect, any strategy that increases the number of metaphase II (MII) oocytes available and, in turn, the number of embryos, is of value in women from whom a low number of mature oocytes are retrieved. Such strategies include the following: the accumulation of vitrified MII oocytes over several stimulation cycles and their insemination at the same time. The goal of assisted reproduction treatment is to transfer an embryo capable of implanting and giving rise to a healthy live newborn. To accomplish this, the most viable embryo in each cohort must be selected for transfer. Nowadays, embryo selection is based on morphological evaluation, morphokinetic characteristics or genetic analysis for chromosomal normality, if performed. Obviously, the developmental competence of a human embryo is determined partly by the correct cytoplasmic and nuclear maturation of the female gamete. Given the complexity of this process, however, it is difficult to find a single factor or characteristic that can be used as an indicator of oocyte competence.

EXPERT MEETING IN PERSONALIZED REPRODUCTIVE MEDICINE

Markus MONTAG

Use of sperm in difficult cases: An update

Markus Montag PhD
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The era of intracytoplasmic sperm injection (ICSI) has facilitated the treatment of couples where the male presents with abnormal semen parameters. Likewise, the range of difficult cases has increased as more male factor patients are treated. This in turn has stimulated the search for therapeutic treatment options that are adapted or tailor-made for these cases. The list of difficult cases comprises 100% immotile sper-matozoa, kryptozoospermia, round-headed spermatozoa, reduced or absent sperm-oocyte activation capacity, incompetent support of embryo development, centriole dysfunction and morphological aber-rations - to mention some but by far not all.

Some can be diagnosed before a fertility treatment is initiated, which allows choosing a proper treat-ment strategy that tries to overcome the fertility issues that are associated with the related diagnosis. However, for other cases a potentially underlying sperm problem is only considered after one or even more than one treatment cycle has failed. Needless o say that the underlying problem is not detected by standard semen analysis. Things get even more complex if the etiology of a difficult sperm case cannot be explained and if it is unclear what is wrong or missing in the sperm cell and if the problem is caused dur-ing spermiogenesis or if it is due to something that alters the fertilization competence after completion of spermatogenesis.

This update will focus on some applications that have been developed in order to support the treatment of difficult cases that are either directly or indirectly related to sperm problems, including oocyte activa-tion, sperm motility enhancement and others.

EXPERT MEETING IN PERSONALIZED REPRODUCTIVE MEDICINE

Sarah MARTINS DA SILVA

Contemporary sperm selection methods: A part of the solution or a part of new problems?

Sarah Martins Da Silva

This presentation considers sperm preparation for ART as well as current technology and methods for sperm selection, including hypo-osmotic selling testing (HOST), hyaluronic acid binding, motile sperm organelle morphology evaluation (MSOME), magnetic activated cell sorting (MACS) and annexin V. How-ever, current tests of sperm selection have consistently failed to provide clear evidence of any real value in improving success rates of ART and a paradigm shift is needed in our understanding of in vivo sperm selection to develop valid in vitro applications. Horizon scanning and future sperm selection technology is considered in this context.

EXPERT MEETING IN PERSONALIZED REPRODUCTIVE MEDICINE

Alex PASTUSZAK

Advanced sperm DNA assessment tools: Why the facts do not fit the theory?

Alexander W. Pastuszak
Assistant Professor of Surgery
Urology in the Department of Surgery at the University of Utah School of Medicine

Contemporary clinical genetic testing for the evaluation of male infertility is limited, and largely restrict-ed to somatic testing that includes karyotype analysis and Y-chromosome microdeletion testing, as well as sperm testing that includes DNA damage testing and fluorescence in situ hybridization (FISH). The lim-itations of these tests are largely their low resolution and their inability to provide a detailed analysis of all defects associated with male infertility. Further, current genetic evaluation of male infertility is lim-ited by a lack of comprehensive knowledge of the DNA, RNA, and epigenetic defects that result in male infertility; to date, only approximately 1,200 genes have been associated with male infertility, encom-passing a large variety of defects that range from single nucleotide changes to structural chromosomal abnormalities. Having a comprehensive understanding of the genetic defects leading to male infertility, which is being made possible through genome-wide, high-throughput sequencing and screening tech-nologies, will facilitate our understanding of the genetic underpinnings of male infertility, and permit the development of high sensitivity and specificity assays to detect the causes of male infertility.

EXPERT MEETING IN PERSONALIZED REPRODUCTIVE MEDICINE

Thomas EBNER

Biological, clinical and technical aspects of poor embryo quality

Univ.-Prof. Mag. Dr. Thomas Ebner
ESHRE certified Senior Clinical Embryologist
Laborleitung Kinderwunsch Zentrum

The term "poor embryo quality" refers to all scenarios in which an embryo shows impaired morpholo-gy/ morphokinetics,developmentaldelayorarrest,irregularorstage-inappropriatecleavageaswellasanyinstance that may affect embryo viability or ploidy. Since controlled ovarian hyperstimulation and subsequent in vitro culture are highly complex processes there is a potential risk that suboptimal treat-ment from clinicians and/ or embryologist may cause the above mentioned situation. In detail, devia-tions in stimulation or ovulation induction may be associated with reduced quality of oocytes, whereas in the lab suboptimal ICSI technique and particularly suboptimal culture conditions (O2, pH, T) will au-tomatically lead to a slump in KPIs since embryonic growth will be affected. Biologically, affected em-bryos will display disturbances in Ca2+-balance and or mitochondrial deficiencies leading either to se-nescence or in more severe cases to apotosis.

ABSTRACTS

TSFP TURKISH SOCIETY FOR FERTILITY PRESERVATION
III. INTERNATIONAL EXPERMED CONGRESS
EXPERT MEETING IN PERSONALIZED REPRODUCTIVE MEDICINE
11-14 APRIL, 2019 | LIMAK CYPRUS DELUXE HOTEL | BAFRA, TURKISH REPUBLIC OF NORTHERN CYPRUS

OP 001

CLOMIPHENE CITRATE VERSUS RECOMBINANT FSH IN INTRAUTERINE INSEMINATION CYCLES WITH MONO- OR MULTI-FOLLICULAR DEVELOPMENT

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ABSTRACT

Objective: The aim of this study was to compare the pregnancy rates of Controlled ovarian stimulation(COS)-IUI(intrauterine insemination) cycles utilizing FSH and Clomiphene citrate in case of both mono-follicular and multi-follicular development.

Methods: A total of 870 infertile patients treated with COS-IUI at a university-based infertility clinic between January 2012 and December 2017 were recruited. Clomiphene citrate and recombinant FSH was administered for ovarian stimulation. HCG was administered when the leading follicle reached ≥18 mm in both groups. A single IUI was performed 36–40 h after hCG injection. Cycles stimulated by CC and rFSH are compared in two set-ups; mono- and multi-follicular development. The main outcome measure was clinical pregnancy rate(CPR) per cycle.

Results: The demographic and cycle parameters were similar between the groups. The overall clinical pregnancy rates in CC and rFSH groups were 9.8% and 10.3%, respectively (P=0.940). The CPR were significantly higher in case of multi-follicular development when compared to mono-follicular development (16.8% vs. 10.2%, respectively; P=0.009). The overall CPR were similar in both mono- and multi-follicular development between the groups.

Conclusion: In the present study we identified that the CPR were similar between IUI cycles stimulated by CC or rFSH, in cases of mono- and multi-follicular development, separately.

1. INTRODUCTION

Controlled ovarian stimulation (COS) combined with intrauterine insemination (IUI) is a common fertility treatment and approved as first line treatment option for unexplained infertility, mild male factor infertility, polycystic ovary syndrome (PCOS) and minimal-mild endometriosis [1-3]. Clomiphene citrate (CC) is mainly accepted as the first line drug in COS-IUI cycles due to its feasibility and cost-effectiveness [4,5]. However, a recent meta-analysis showed that FSH(follicle-stimulating hormone) is superior to CC regarding clinical pregnancy rates [6]. Although the primary aim is to achieve a monofollicular growth in COS-IUI cycles, previous studies suggested significantly higher pregnancy rates with multifollicular development [7,5]. However, several studies failed to show a significant difference between one and two preovulatory follicles in terms of multiple pregnancy rates [8,7].

The aim of this study was to compare the pregnancy rates of COS-IUI cycles utilizing FSH and CC in case of both mono-follicular and multi-follicular development.

2. MATERIALS AND METHODS

Infertile patients treated with COS-IUI at a university-based infertility clinic between January 2012 and December 2017 were recruited fort his retrospective study. All couples had a standard infertility work-up including medical history, physical examination, semen analysis (abnormal semen test results confirmed by a second analysis 6 weeks later), HSG to confirm tubal patency, and basal hormone profile.

A baseline ultrasound exam was performed on cycle day 2 or 3 before starting a cycle. Clomiphene citrate (Klomen; Kocak Farma, Istanbul, Turkey) was administered at a 100 mg daily dose for five days starting from cycle days 3-5. Recombinant FSH (Gonal-F; Merck-Serono, Istanbul, Turkey or Puregon; Organon, Istanbul, Turkey) was administered according to body mass index (BMI). Starting rFSH dose was 75 IU/day for women with BMI <25 kg/m2 and 100 IU/day for women with BMI >25 kg/m2. The first monitoring scan was performed on the 6th day of gonadotrophin stimulation, and repeated after 2 or 3 days, depending on the follicular growth. HCG (Pregnyl; Schering-Plough, Istanbul, Turkey) 10,000 IU was administered when the leading follicle reached >18 mm in both groups. A single IUI was performed 36–40 h after hCG injection. Semen samples were taken by masturbation and collected in sterile containers. Semen samples used for insemination were processed within one hour after ejaculation, using a density gradient centrifugation followed by washing with culture medium. No luteal phase support was given.

The first study group consisted of patients stimulated by CC and the second study group consisted of patients stimulated by rFSH. The success rates of the groups were compared under two separate conditions; in case of mono-follicular development or in case of multi-follicular development. Multi-follicular development was defined as at least two dominant follicles reaching minimum 17 mm in diameter. Pregnancy was defined by positive serum β-hCG levels 2 weeks after IUI. Clinical pregnancy was defined as the presence of heartbeat at 6th gestational weeks. The primary outcome measurement was clinical pregnancy rate (CPR)

2.1. Statistical Methods

Data analyses were performed by using SPSS Version 21.0 (IBM Corporation, Armonk, NYC, USA). Samples were tested with Kolmogorov-Smirnov test to determine normality of distributions. Continuous variables were compared with Student's t test (for normally distributed data) and Mann-Whitney U test (for skewed data). Categorical variables were compared with Chi-square test. Multivariate logistic regression analysis with a model building strategy was used to determine independent predictors of clinical pregnancy in IUI cycles. A P value of <0.05 was considered statistically significant.

3. RESULTS

A total of 1081 cycles of 682 couples were assessed for eligibility. The cycles stimulated other than CC or rFSH were excluded as well as the cycles in which three or more follicles developed. As a result, the final analyses included 870 cycles of 590 couples. The groups were mainly similar to each other according to the demographic parameters. The female age and the duration of infertility were significantly higher in the rFSH group than the CC group (P=0.003 and P=0.009, respectively). As expected, endometrial thickness on the day of hCG was significantly lower in the CC group than the rFSH group due to the anti-estrogenic effect of CC (7.77±2.12 vs. 9.73±2.33, respectively; P=0.001). The overall clinical pregnancy rates in CC and rFSH groups were 9.8% and 10.3%, respectively (P=0.940). Regarding the entire cohort, the CPR were significantly higher in case of multi-follicular development when compared to mono-follicular development (16.8% vs. 10.2%, respectively; P=0.009).

The overall CPR were similar in both mono- and multi-follicular development between the groups(p=0.847 and p=0.701 for mono- and multi-follicular, respectively). In addition, the subgroup analyses according to the cause of infertility showed no statistically significant differences between the groups.

A multi-variate analysis was performed to identify the factors which has impact on CPR. According to the results, multi-follicular development and endometrial thickness on the day of hCG significantly increased the odds of clinical pregnancy. Odds ratios of multi-follicular growth and endometrial thickness were 2.459(95 CI, 1.213–4.983, p=0.013) and 1.177(95 CI, 1.031–1.344, 0.016), respectively. However, no other factors had a significant influence on the success rate of an IUI cycle.

4. CONCLUSION

In the present study we identified that the CPR were similar between IUI cycles stimulated by CC or rFSH, in cases of mono- and multi-follicular development, separately. We also determined that multi-follicular development and increment in endometrial thickness increased the likelihood of clinical pregnancy in COS-IUI.

Multi-follicular development has a significant impact on IUI cycles, and CC and rFSH cycles result in comparable rates of multi-follicular development. In any indication, CC and FSH has similar success in terms of CPR, in either mono-follicular development or multi-follicular development.

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OP 002

A FEASIBLE OPTION BEFORE CYCLE CANCELLATION FOR WOMEN UNRESPONSIVE TO CONTROLLED OVARIAN STIMULATION DESPITE MAXIMUM DOSE GONADOTROPHINS; STOP AND RE-START IN THE SAME CYCLE

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ABSTRACT: Despite the advances in controlled ovarian stimulation (COS), management of a subgroup of poor responders may still be challenging. Herein, we describe a feasible and simplified protocol, Stop and Re-start, for Bologna poor responders who are unresponsive to COS. Data of 11 women unresponsive to COS were reviewed (n=11). The mean age of the cohort was 36.5±6.0 years. Unresponsiveness was defined as no follicular growth >9 mm and/or the estradiol level less than 40 pg/ml after a week of rFSH administration. In that case, COS was stopped and they were followed-up weekly. All women showed at least one follicular growth within five to 20 days. Six women (54.5%) had spontaneous follicular growth and the other five required ovarian stimulation. At least one oocyte was retrieved from seven patients (63.6%). The mean number of oocytes retrieved was 1.6±1.4 and five women (45.5%) had at least one Grade A embryo. We achieved two ongoing pregnancies, all in the cohort (18.2%). In conclusion, Stop and Re-start protocol is an effective management option for Bologna poor responders who are unresponsive to COS.

Key words: Bologna criteria; poor response; stop-start protocol; unresponsive,

1. INTRODUCTION

Despite the advances in controlled ovarian stimulation (COS) protocols the management of poor responder patients is still challenging. Recently, the POSEIDON (Patient-Oriented Strategies Encompassing IndividualizeD Oocyte Number) group developed a detailed classification system to better identify the poor ovarian response patients who would be included in future studies investigating diagnosis and management (1). Several researches have been held and strategies have been developed to improve the success in assisted reproductive technology (ART) cycles of poor responder patients. However, there's still a subgroup of poor responders who requires important decision making; unresponsive patients to COS. The aim of the present study is to assess the preliminary results of a novel protocol, stop-start protocol, for patients who are unresponsive to COS.

2. MATERIALS AND METHODS

Data of poor responder patients who underwent COS and were unresponsive to stimulation at a university-based infertility clinic between July 2017 and July 2018 were reviewed. Bologna poor responders who were unresponsive to COS and managed by Stop-Start protocol were selected from the hospital database. The inclusion criteria were female age 18–45 years, fulfilling the Bologna criteria (2), a starting dose of gonadotrophin stimulation with 225-300 IU/day, and unresponsive to first COS. The exclusion criteria were presence of any untreated thyroid dysfunction or hyper-prolactinaemia. Eleven patients were found to be eligible for analyses and all data regarding COS and clinical outcomes were extracted from the database. Unresponsiveness was defined as no follicular growth >9 mm and/or the estradiol level less than 40 pg/ml after a week of 225-300 IU/day rFSH (Gonal-F; Merck-Serono, Istanbul, Turkey) administration. Then, the rFSH was stopped and patients were called for a weekly follow-up to begin after one week (STOP period). When a new wave of follicular growth gets caught the patients were then accepted into ART cycle (START period). The patients were then followed-up spontaneously or under ovarian stimulation. The primary outcome measure was ongoing pregnancy rate.

3. RESULTS AND DISCUSSION

The mean age of the patient cohort was 36.5±6.0 years and all patients fulfilled the Bologna criteria (Table 1). All of the patients were unresponsive to a standard initial COS and all patients showed at least one follicular growth within five to 20 days after stopping gonadotrophins. Six patients (54.5%) had spontaneous follicular growth and the other five required ovarian stimulation. We were able to retrieve at least one oocyte from seven patients (63.6%). The mean number of oocytes retrieved was 1.6±1.4 and there were five patients (45.5%) with at least one Grade A embryo. As a result, we have achieved two ongoing pregnancies, all in the cohort (18.2%) (Table 1). The first pregnancy was a spontaneous one following oocyte retrieval. The patient was 35 years old and her AMH level was 0.68 ng/ml (POSEIDON Group 4). Following Stop-Start protocol we retrieved two MII oocytes and both were fertilized. She had one frozen Grade A embryo and she was called for endometrial preparation on the 2nd day of next cycle. However, she referred 20 days later with menstrual delay and her βhCG test was positive. The second pregnancy was achieved following a fresh embryo transfer in a 29-year-old woman whose AMH level was 0.09 ng/ml (POSEIDON Group 3). Following Stop-Start protocol we retrieved three oocytes in which only one was MII. Although we opt frozen-thawed embryo transfer, fresh embryo was transferred by patient demand and in the light of first patient.

Recently, the wave theory was proposed suggesting that the follicles may be recruited two to three times within a single menstrual cycle, even in the luteal phase. Corresponding to this theory we waited for the next follicular wave of the patients. The other novel protocols identified by wave theory are random-start COH and double stimulation (DuoStim) [3-6]. The main difference of Stop-Start protocol from DuoStim is the long drug-free interval of approximately one week.

Theoretically, a hormone-receptor complex may be deactivated by external shedding or by internalization of the receptors into the cell. Excess concentrations of tropic hormones, such as FSH, stimulate the process of internalization, leading to a loss of receptors in the cell membrane and a decrease in biologic response (7). During a standard COS daily, high dose recombinant FSH is utilized to stimulate the present follicular cohort. The high dose FSH administration in a non-pulsatile manner might down-regulate the previously reduced receptors by internalization, and at this point the patient becomes unresponsive. However, cessation of pushing (STOP period) probably prevents down-regulation of FSH receptors and allows development of a new follicular cohort (START period).

Table 1. The cycle characteristics of the study population.

		Patients (N=11)	Mean±SD	Min-Max
DEMOGRAPHICS	Age (years)	36.5±6.0	25-45	
	Body mass index (kg/m^2)	25.6±4.3	22-36.8	
	Baseline E_2 (pg/ml)	46.9±30.1	20-113	
	Baseline FSH (IU/ml)	18.5±8.2	9-36	
	Baseline AMH (ng/ml)	0.23±0.25	0.01-0.68	
	Antral follicle count	2.4±1.6	1-6	
	Duration of infertility (years)	5.0±3.5	1.5-12	
	Number of previous IVF attempts	1.3±1.0	0-3	
1-COH	Duration of stimulation (days)	7.9±2.3	5-12	
	Total dose of gonadotrophins (IU)	1955±1033	900-3600	
	E_2 levels on the day of cancellation (pg/ml)	31.6±10.4	17-42	
2-STOP	Duration of cessation period (days)	9.3±4.5	5-20	
	Number of follicles >9 mm at return	2.4±0.9	1-4	
	E_2 levels at return (pg/ml)	178.9±100.5	41-390	
3-SECOND WAVE	Number of patients with spontaneous follow up (%)	6 (54.5)		
	Maximal E_2 levels at follow-up (pg/ml)	332.0±91.9	193-445	
	Retrieved oocytes (n)	1.6±1.4	0-3	
	Number of MII oocytes	1.1±1.1	0-3	
	Fertilization rate (%)	64.2±39.0	0-100	
	Number of Grade A embryos	0.9±0.9	0-2	
	Number of patients with at least one Grade A embryo (%)	5 (45.5)		
	Ongoing pregnancy, n (%)	2 (18.2)		

4. CONCLUSION

Stop-Start protocol is an effective management option for Bologna poor responders who are unresponsive to COS. However, it's necessary to prove the feasibility of Stop-Start protocol by further prospective trials.

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OP 003

THE DETERMINATION OF ANTI-MULLERIAN HORMONE AND VITAMIN D SERUM LEVELS IN POLYCYSTIC OVARY SYNDROME

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ABSTRACT: The aim of this study was to determine whether there is a relationship between serum anti-mullerian hormone (AMH) and vitamin D levels and the severity of policystic ovary syndrome. Forty four women with polycystic ovary syndrome (PCOS) and forty four controls with normal ovulatory menstrual cycles were included in this study between Fabruary, 2016 and November, 2016. Hormonal parameters, glucose metabolism parameters, clinical signs and symptoms and serum AMH and vitamin D levels were determined. AMH levels, luteinizing hormone (LH) levels, LH/ follicle stimulating hormone (FSH) ratio, total testosterone, dehydroepiandrosterone sulfate (DHEA-S), hirsutism scores, and postprandial glucose levels were significantly different between two groups. There were statistically significant positive correlations between AMH and LH/FSH ratio, homeostatic model assessment of insulin resistance (HOMA-IR), fasting glucose levels and hirsutism scores. Serum AMH levels were significantly higher in women with PCOS compared to controls and the patients with higher AMH levels were more hyperandrogenic compared to the patients with PCOS who have lower AMH levels.

Key words: Anti-mullerian hormone; Hirsutism; Hyperandrogenism; Polycystic ovary syndrome; Vitamin D

1. INTRODUCTION

Policystic ovary syndrome (PCOS) is the most common endocrine disorder in women during the reproductive ages and is often accompanied by insulin resistance and hyperinsulinemia [1].

Recently there has been a focus on vitamin D supplementation as an adjuvant treatment of PCOS. Indeed, women with PCOS have been found to have a high prevalence of vitamin D deficiency. Additionally, some studies have found a correlation between serum vitamin D levels and several metabolic symptoms in women with PCOS such as type 2 diabetes mellitus [2,3].

To date, several clinical trials have evaluated the effects of vitamin D on women with PCOS. There is some, but limited, evidence for beneficial effects of vitamin D supplementation on insulin resistance, ovarian follicles maturation, ovulation and menstrual regularity in women with PCOS [4,5].

Women with PCOS have high concentrations of anti-mullerian hormone (AMH) [6]. AMH is a glycoprotein produced in the granulosa cells of the ovary that regulates early follicular recruitment [7]. It is secreted by preantral and small antral follicles, and there is a good correlation between AMH and ovarian follicle count [8]. Recent studies focus on the determination of the relationship between AMH and PCOS as well as the clinical utilization of serum AMH as an adjunct test in the diagnosis of PCOS [9]. In a recent meta-analysis, symptomatic PCOS patients have serum AMH levels higher than 4,7 ng/ml [6].

The aim of this study is to determine serum vitamin D and AMH levels in PCOS patients and to investigate the correlation between serum levels of these two factors and the severity of the syndrome.

2. MATERIALS AND METHODS

Forty-four (44) women with PCOS and 44 age- body mass index (BMI) matched controls were enrolled in this study between February and November 2016.

The diagnosis of PCOS was considered based on the presence of at least two criteria of Rotterdam: (i) oligo- and/or anovulation, (ii) the presence of the clinical and/or biochemical markers of hyperandrogenism, (iii) polycystic ovaries in ultrasonography (USG). The inclusion criteria were: (i) the women between 18-40 years old, (ii) BMI ≤ 35 kg/m^2 (ii) absence of systemic and/or metabolic disease, (iii) absence of drug use in the last 3 months that will affect hormonal and/or insulin metabolism. Women with thyroid dysfunction, hyperprolactinemia and chronic systemic disorder such as type 1 or 2 DM or hypertension were excluded from this study.

Day 3 serum follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E_2), prolactine, thyroid stimulating hormone (TSH), total testosterone, free testosterone, 17-hydroxyprogesterone (17-OH-P), dehydroepiandrosterone sulfate (DHEA-S), vitamin D, and AMH levels were analyzed. Serum levels of fasting glucose, postprandial glucose, fasting insulin, and HbA1C were determined. Ferriman- Gallwey scoring system was used to detect hirsutism and ≥ 8 is defined as hirsutism [10]. Insulin resistance (IR) was determined with homeostasis model assessment for IR (HOMA-IR) as: HOMA-IR = fasting glucose (mg/dL) x fasting insulin (µIU/ml)/405 [11].

3. RESULTS AND DISCUSSION

The hirsutism score was significantly higher in women with PCOS than controls (10 vs 3, respectively). Day 3 serum LH levels and LH/FSH ratio, total testosterone and DHEA-S levels were higher in women with PCOS than controls (6.65 mIU/ml vs 3.6 mIU/ml for LH, 1.3 vs 0.58 for LH/FSH ratio, 30.7 ng/dl vs 13.6 ng/dl for total testosterone, and 244.4µg/dl vs 204.68 µg/dl for DHEA-S, respectively). There was no statistically significant difference regarding to serum fasting glucose, fasting insulin and HbA1C, only postprandial glucose levels was higher in women with PCOS than controls (92 mg/dl vs 86.5 mg/dl, respectively). AMH levels was higher in women with PCOS than controls (5.9 ng/ml vs 2.7 ng/ml, respectively), but there was no statistically significant difference in serum vitamin D levels between two groups. Vitamin D deficiency is also common in the general population in many parts of the World, with 10-60% of adults having values lower than 20 ng/ml [12]. In our study, the median value of vitamin D levels in PCOS patients was 12.1 (5.8-20.3) ng/ml and in control group it was 11.6 (8.2-17.4) ng/ml and it is noteworthy that the median vitamin D levels were low in both groups.

The exact mechanism underlying the association of vitamin D and insulin resistance is not known yet. Wehr, et al. reported that the level of 1.25OHD was a significant and independent predictor for HOMA-IR and BMI by using multivariate regression analysis [2]. In our study, we found a negative correlation between vitamin D levels and HOMA-IR in women PCOS (R= -0.3).

There were statistically significant positive correlations between AMH and hirsutizm score, LH/FSH ratio. It has been also hypothesised that AMH is positively correlated with serum androgens. While the severity of hyperandrogenism is correlated with the severity of ovulatory disturbance, hyperandrogenism is suspected to increase the AMH production by promoting an excess of small growing follicles and granulosa cell proliferation [13,14].

Tokmak, et al. [15] studied the association between serum AMH levels and IR in non-obese adolescent females with PCOS either with IR or without IR. They reported that there was a significant positive correlation between serum AMH and HOMA-IR levels in PCOS patients. We also found similar results regarding the correlation between AMH and HOMA-IR (p=0.03).

4. CONCLUSION

There were statistically significant positive correlations between AMH and hirsutism score, LH/FSH ratio, fasting glucose levels and HOMA-IR. There was a negative correlation between vitamin D levels and HOMA-IR in women with PCOS.

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OP 004

DECREASED BMP-15 AND BDNF MRNA EXPRESSİON LEVELS İN CUMULUS GRANULOSA CELLS OF WOMEN WİTH OVARİAN ENDOMETRİOSİS

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ABSTRACT: The aim of this study was to evaluate the expression pattern of *BMP-15* and *BDNF* genes in cumulus granulosa cells from mature (metaphase II) oocyte of women with ovarian endometriosis. Twenty (20) women with ovarian endometriosis and 20 controls who underwent ovarian stimulation for intracytoplasmic sperm injection enrolled in this study. The diagnosis of the endometrioma was performed using transvaginal ultrasonography, and endometrioma had to be documented on at least two occasions and at least two menstrual cycles apart. The control group consisted of patients with infertility purely due to a male factor (azoospermia or severe oligoasthenoteratozoospermia). There was no significant difference in the basal conditions between women with endometriosis and controls, such as age, body mass index, basal follicle stimulating hormone and estradiol levels, and total gonadotrophin dosage. The *BMP15* and *BDNF* mRNA levels in cumulus granulosa cells of patients with ovarian endometriosis were statistically significantly decreased than controls (fold changes: -3.99 and -2.15, respectively). In endometriosis, a decreased mRNA level of *BMP15* and *BDNF* in cumulus granulosa cells could be one of the mechanism that adversely affect folliculogenesis and oocyte quality in this women.

Key words: BDNF; BMP15; cumulus cells; gene expression; ovarian endometriosis

1. INTRODUCTION

Endometriosis is benign, invasive gynecological disase, defined by the presence of endometrial glandular epithelial and stromal cells outside the uterus [1]. Endometriosis is a common disease; it affects an approximately 5-15% women in reproductive age group. It is fortunate that not all women who have endometriosis are infertile, but endometriosis has much higher prevalence in infertile women, estimated at between 30 and 40% [2]. Although a definite causal relationship has not been confirmed, endometriosis is associated with infertility, but the mechanism by which endometriosis may cause infertility is not clearly understood. The fecundity rate in fertile couples is estimated to be approximately 15-20%, while the fecundity rate in women with endometriosis is estimated to be around 2-10% [3]. Studies on assisted reproductive technology (ART) have suggested that endometriosis have been reported to have a poor ovarian reserve, low oocyte and embryo quality [4]. Some studies have reported that fertilization, implantation and pregnancy rate are lower in women with endometriosis than controls that undergo ART [5]. Owing to similar implantation rates in oocyte donation cycles have been reported between women with endometriosis and controls, this result suggesting the critical role of oocyte quality in diminished IVF outcome of women with endometriosis [6].

Oocyte maturation is under precise regulatory control, not only from intrinsic cellular processes, but also from extrinsic influences. Cumulus granulosa cells (CCs), which are closely associated with the oocyte play a important role in the maturation and acquisition oocyte competence [7]. Disruption or deregulation of the CC interactions with the oocyte can affect the quality of oocyte and consequently embryo. Investigation of CCs is likely to reveal key information concerning the viability and genetic structure of their connected oocyte, also increase understanding of normal follicular development and the impact of diseases.

Gene expression has a central role in the regulation of nearly every aspect of cellular life. Quantification of gene transcripts can provide a sign of the several processes taking place within a cell, and may expose the basis of biological problems [8]. In this study we evaluated two genes, *Bone Morphogenetic Protein 15 (BMP15)* and *Brain-Derived Neurotrophic Factor (BDNF)*, which are they play an important role in ovarian function, including follicular development, nuclear and cytoplasmic maturation of oocyte, ovulation, and early embryo development [9, 10].

The aim of this study was that whether the *BMP15* and *BDNF* gene expression in CCs from metaphase II oocytes of patients with endometrioma is changed in patients with ovarian endometriosis.

2. MATERIALS AND METHODS

Twenty (20) women with ovarian endometriosis and 20 controls who underwent ovarian stimulation for intracytoplasmic sperm injection were recruited in this study.

The diagnosis of the endometrioma was performed using transvaginal ultrasonography, and endometrioma had to be documented on at least two occasions and at least two menstrual cycles apart. The control group consisted of patients with infertility purely due to a male factor (azoospermia or severe oligoasthenoteratozoospermia). Women with a history of the following procedures or disorders were excluded: ovarian surgery, radiotherapy or chemotherapy, premature ovarian failure, polycystic ovarian syndrome, hyperprolactinemia, thyroid dysfunction or ovulation induction within 3 months. Women with infertility due to poor ovarian reserve, ovulatory dysfunction, tubal factor, and endometriosis were excluded from the controls.

The patients received a standard gonadotropin-releasing hormone antagonist regimen starting on day 6 of a spontaneous menstrual cycle. Oocytes were collected using transvaginal ultrasonography-guided needle aspiration of the follicles for 36 hours after human chorionic gonadotropin administration. Cumulus-oocyte complex (from one large pre-ovulatory follicle) retrieval was performed by vaginal puncture under ultrasound echo-guidance 36 h after hcG administration. Immediately after identification of COC of the follicle aspirated, CC were separated from the oocytes by mechanically in sterile conditions, washed in culture medium, and then placed within a cryotube within RNA later and were immediately frozen in liquid nitrogen and stored -80 until RNA extraction. Only cumulus cells of metaphase II oocytes were analyzed. Quantitative RT-PCR (qRT-PCR) were used to analyze the expression of *BMP15* and *BDNF* genes in CCs

3. RESULTS AND DISCUSSION

There was no statistically significant difference between women with endometriosis and controls regarding to age and body mass index (BMI) (Table 1). The basal follicle stimulating hormone (FSH) and estradiol (E_2) levels, and total gonadotrophin dose are similar between women with endometriosis and controls (Table 1). There was not any statistically significant difference between the groups in terms of the number of retrieved oocytes, MII oocytes, number of embryos, and success of clinical pregnancy (Table 1).

Table 1. Clinical Characteristics of Study Participant's

Variables	Women with endometrioma n=20	Controls n=20	*p*
Age	33.7±3.1	31.5±5.9	0.18
BMI	23.4±2.6	25.0±3.1	0.10
D3 FSH (mIU/mL)	8.7±4.3	6.9±1.7	0.21
D3 LH (mIU/mL)	6.1± 3.0	5.2±1.7	0.26
D3 E_2 (pg/mL)	47.1±18.8	47.8±24.6	0.93
Total gonadotrophin dose	2227±739	2204±1146	0.94
Number of oocyte retrieved	7.1±4.6	10.6±3.0	0.08
Number of mature oocyte	4.6 ±3.4	7.1±2.9	0.07
Number of embryos produced	4.2±1.92	3.12±0.83	0.19
Clinical pregnancy rate	6/14 (30%)	5/15 (25%)	0.72

The *BMP15* and *BDNF* mRNA levels in CCs of patients with ovarian endometriosis were statistically significantly decreased than controls (fold changes: -3.99 and -2.15, respectively).

In the present study, we found that *BMP-15* mRNA levels in CCs of patients with ovarian endometriosis were significantly decreased than controls. *BPM-15* has been expressed in the mammalian ovary (both oocytes and CCs) [11]. In an animal study, it has been revealed that BMP15 can stimulate oocyte development [12]. *BMP-15* mRNAs in CCs was positively associated with oocyte maturation, normal fertilization rate, and cleavage rate of patients undergoing ART [10].

In the present study, we found that *BDNF* mRNA levels in CCs of patients with ovarian endometriosis were significantly decreased than controls. *BDNF* is expressed in oocytes and CCs, but it's secretion predominantly from CCs [13]. In agreement with this study, Buyuk and Seifer reported that women with a history of endometriosis had significantly lower follicle fluid BDNF levels compared with male infertility [14]. In

the preovulatory period, *BDNF* is rapidly induced after the LH surge and its play important role for finally follicle maturation and ovulation [15]. In addition to, Kawamura et al. [10] had found that BDNF enhanced the first polar body extrusion of oocytes as well as cytoplasmic maturation of oocyte, which important for early embryo development

4. CONCLUSION

In endometriosis, a decreased mRNA level of BMP15 and BDNF in cumulus granulosa cells could be one of the mechanism that adversely affect folliculogenesis and oocyte quality in this women.

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OP 005

A RETROSPECTIVE COMPARISON OF GnRH ADMINISTRATION IN ADDITION TO hCG FOR OVULATION TRIGGERING WITH STANDARD hCG TRIGGERING REGARDING CLINICAL PREGNANCY OUTCOMES IN COMBINED INTRAUTERINE INSEMINATION CYCLES WITH GONADOTROPIN

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ABSTRACT **A standard single dose 250 mcg recombinant human chorionic gonadotropin (hCG) is administered for final follicular maturation and ovulation triggering, after ovulation induction with gonadotropins for intrauterine insemination (IUI) cycles. There are many studies that compares the combined use of 0.2 mg GnRH agonist and hCG with standard hCG regarding ovulation triggering, and it appears that combined use of GnRH and hCG triggering is associated with increased clinical pregnancy rates than standard hCG triggering. We aimed to establish the relationship between the aforementioned combined ovulation triggering and the clinical pregnancy and OHSS outcomes in gonadotropin-induced IUI cycles. There were no differences between the groups in terms of clinical and biochemical pregnancy outcomes. Concerning clinical pregnancies and outcomes of OHSS, more prospective randomized studies with larger number of cases are needed.**

Keywords: Intrauterine insemination, OHSS, triggering, GnRH agonist.

1.INTRODUCTION

A standard single dose 250 mcg recombinant human chorionic gonadotropin (hCG) is administered for final follicular maturation and ovulation triggering, after ovulation induction with gonadotropins for intrauterine insemination (IUI) cycles. hCG has the same effect with the long half-life luteinizing hormone (LH). It has a long luteotropic effect that increases the risk of ovarian hyperstimulation syndrome (OHSS). According to recent studies regarding IVF cycles, gonadotropin relasing hormone (GnRH) agonists are used for final follicular maturation and induction of ovulation in order to prevent OHSS. When the ovulation is triggered with GnRH analogue, the peak of FSH (follicle stimulating hormone) imitates the natural cycle. FSH increases the LH receptors on the granulosa cells for ovulation and improves the maturation of the oocyte nucleus. Concurrently, it contributes to the maturation of the oocyte nucleus by providing the endogenous increase of LH. There are many studies that compares the combined use of 0.2 mg GnRH agonist and hCG with standard hCG regarding ovulation triggering, and it appears that combined use of GnRH and hCG triggering is more associated with increased clinical pregnancy rates than standard hCG triggering. There are many prospective randomized controlled studies concerning IVF cycles. We aimed to establish the relationship between the aforementioned combined ovulation triggering and the clinical pregnancy and OHSS outcomes in gonadotropin-induced IUI cycles in our retrospective study.

2. MATERIALS AND METHODS

Our study was conducted with cases of primary infertility at the infertility clinic of Dokuz Eylul University School of Medicine. The patients had visited the clinic between October 2017 and October 2018, and their planned treatment was intrauterine insemination combined with gonadotropin administration. Before the initiation of the study, ethical approval of Dokuz Eylül University Non-Invasive Research Ethics Committee had been obtained. The total number of included cases is 74: Group I (standard dose 250 mcg recombinant human chorionic gonadotropin) n=38; Group II (in addition to hCG injection, administration of 0.2 mg GnRH analogue 2 hours later); n: 36. According to the case files, ovulation induction with gonadotropins was administered as follows: Daily subcutaneous injection was performed using recombinant FSH and patientspecific, with individualized planned classical or low-dose step-up protocols. Standard semen parameters were evaluated according to the 2010 guidelines of WHO, morphological examination was analyzed in the laboratory of the Department of Obstetrics and Gynecology, Dokuz Eylul University School of Medicine according to Kruger criteria. According to the case files, the same person prepared the samples for insemination. In both groups, 32-36 hours after the ovulation triggering with hCG, intrauterine insemination was performed. After intrauterine insemination, all patients rested in supine position for 10 minutes. The groups received 600 mg of micronized progesterone, applied intravaginally, divided into three capsules per day, for luteal phase support. Two weeks after the insemination, the patients were tested for pregnancy by serum ß-hCG measurement. Patients with serum ß-hCG values above 25 mIU/mL were evaluated as biochemical pregnancies. The presence of clinical pregnancy was defined as the detection of fetal heart beats on transvaginal ultrasound 3 weeks after ß-hCG value positivity. Medison SonoAce X6 C3-7EP, transvaginal ultrasound probe C2-8 was used for follow-up. Blood samples were collected from Central Laboratory of Dokuz Eylül University School of Medicine and the results were evaluated.

3. RESULTS AND DISCUSSION

Results of 74 couples were examined. Group I consisted of 38 cases, while group II had 36 cases. The mean age of the female patients was 28.9 (20-35) and the mean age of the male patients was 33.2 (22-47). Male smoking habit (package / year) and female smoking habit (package / year) were similar in both groups according to patient histories. Similar results were obtained in terms of history of infertility treatment in both groups. The groups were similar in terms of female age, male age, body mass index (BMI) and duration of infertility. No difference was observed between the groups in terms of infertility. Male factor was the most common cause of infertility in both groups. There was no difference between the groups in terms of basal serum hormone levels. While evaluating intrauterine insemination cycles, stimulation time (day), total gonadotropin dose (IU/day) and triggering day endometrial thickness (mm) criteria were evaluated. There was no difference between the groups in terms of these criteria. There was no difference in sperm parameters between the groups. Both biochemical pregnancy and clinical pregnancy rates were %13.2 in Group I and %27.8 in Group II, but there was no statistical difference between the groups (p= .11). Dichorionic diamniotic multiple pregnancy was detected clinically in 2 cases in Group I (p= .16). Group II had no multiple pregnancies. No cases of OHSS was present in the both groups.

4. Tables

Table 1. . Demographic characteristics of patients

	Group I (hCG trigger n:38				Group II (hCG+agonist trigger) n: 36				*P value*
	Min.	Max.	Mean	±SD	Min.	Max.	Mean	±SD	
Female age (year)	22	35	28.97	3.95	20	35	28.94	4.52	.48
Male age (year)	27	47	34.05	4.27	24	46	32.30	5.90	.16
BMI (%, kg/m²)	18	35.5	26.10	4.63	18.60	33	25.5	3.67	.56
Infertility duration (year)	1	11	3.73	2.06	1	10	2.88	2.88	.20
Causes of infetility n, %									.81
Unexplained	5, 13.2 %				5, 13.9 %				
PCOS	4, 10.5 %				5, 13.9 %				
Male factor	22, 57.9 %				21, 58.3 %				
Endometriosis	1, 2.6 %				0, 0 %				
Tubal factor	6, 15 %				5, 13.9 %				

Table 2. Basal hormone levels

	Group I (hCG trigger n:38				Group II (hCG+agonist trigger) n: 36				*P value*
	Min.	Max.	Mean	±SD	Min.	Max.	Mean	±SD	
FSH (mIU/mL)	3.56	10	7.59	1.47	4.46	10	6.99	1.69	.29
LH (mIU/mL)	2.26	42.44	8.24	6.67	2.36	93	8.33	14.64	.38
E_2 (pg/mL)	5.2	60	32.4	2.78	1.9	60	36.99	21.24	.06
PRL (ng/mL)	2.10	20.60	11.24	4.37	1.06	94	15.78	15.82	.24
TSH (µIU/mL)	1.03	2.96	1.82	0.48	0.71	2.90	1.66	0.47	0.79

Table 3. Comparison of IUI cycle characteristics

	Group I (hCG trigger *n:38*				Group II (hCG+agonist trigger) *n: 36*				*P value*
	Min.	Max.	Mean	±SD	Min.	Max.	Mean	±SD	
Stimulation (day)	6	21	10.0	3.27	5	19	10	3.48	.47
Total gonadotropin dose	400	183.50	712.50	322.90	375	1425	675	249.47	.35
Endometrial thickness on trigger day	6.50	15	10	2.03	6	13	9.15	1.47	.06

Table 4. Pregnancy outcomes of IUI cycles

	Group I (hCG trigger) *n:38*	Group II (hCG+agonist trigger) *n: 36*	*P value*
Pregnancy n, %	5, 13.2 %	10, 27.8 %	.11
Clinical pregnancy n, %	5, 13.2 %	10, 27.8 %	.11
Multiple pregnacy n, %	2, 5.3 %	0, 0 %	.16

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OP 006

THE EFFECT OF MİLD EXERCİSE ON TESTİCULAR TİSSUE İN DİABETES RATS

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ABSTRACT

Aim: Diabetes is a metabolic disorder effecting most of the world's population causes damage in many tissue and organs in both early and late stages. It is already known that in diabetic patient there is serious damage in testes where sperms are produced. Therefore, we have does this study, streptozotocin (STZ) diabetic rats with the literature, which has been shown that effects of exercise slow application of testicular tissue, the possible positive effects of exercise and were investigated histologically changes.

Material Method: In our study, we used 30 Sprague Dawley male rats. We have formed three different groups including control, diabetes and diabetes + mild exercise. Mild exercise group started exercise 3 days ago without diabetes. After 3 days, a single dose of 40 mg/kg streptozotocin was administered and exercise was performed until the end of the experiment. At the end of four weeks, the rats testicular tissues were obtained to perform histological stains. The morphometric tests were: testicular weights, blood glucose levels and daimeters of seminiferous tubule.

Results: The findings in our study showed that diabetes groups reduction in testicular weights. The testis tissue of control group was normal, whereas in the testis tissue of diabetes group, primary spermatocytes and spermatids were observed in most tubules despite atrophic changes. It was seen that some cells were separated from the basement membrane and discharged into the lumen. In the mild exercise group, most of the seminiferous tubules were found to be close to normal structure and there were no significant difference in microscopic direction with the control group.

Conclusion: In our study, it was supported in our findings that exercise had significant effect on infertility which is a complication of diabetes. Molecular studies on this subject are needed in the future.

Key words: Diabetes mellitus, male infertility, testis degeneration, rat, streptozotocin

INTRODUCTİON

Diabetes mellitus (DM) is a systemic chronic metabolic disease with hyperglycemia, dyslipidemia, glucosuria and many accompanying clinical and biochemical findings [1]. There are millions of people in the world who are influenced by DM. Deaths occur in many countries due to complications of DM [2]. The number of studies on the etiology, diagnosis and treatment of the disease is increasing day by day since it causes a great economic loss due to the significant morbidity, mortality and treatment. Long-term diabetic complications negatively affect the functions of many tissues, organs and systems [3,4]. Diabetes is associated with reproductive disorders in both men and women. The percentage of sperm that is quite high immature and destined for apoptosis, owner low mobility, abnormal acrosoma and morphology in diabetics [5]. Diabetes decreased testosterone level and weakened spermatogenesis, changes in sperm number and motility, decrease in testis weight, tunica albuginea of testes, seminiferous tubules, Sertoli cells, interstitial tissue and Leydig cells histological changes are common findings in men with diabetes. Studies conducted so far have shown that diabetic testicular tissue increases apoptosis and due to spermatogenesis deteriorates [6].

It is known that exercise has an important role in the treatment of diabetes. Physical activity reduces the risk of cardiovascular disease and diabetes, and there are also important benefits for many conditions, except those related to obesity [7]. Exercise is still a neglected aspect in the treatment of patients with type 2 diabetes, especially in the early period where is dominant insulin resistance. A minimum of 30 minutes of mild exercise is recommended for 4-5 days of the week. When regular exercise is performed next to insulin therapy [8].

In this study; in experimental diabetes to male rate testis tissues induced STZ, depending on the complications of DM; the aim of this study was to determine the effect of exercise on negative results in infertility, testicular sperm count, sperm motility and testicular weight change.

MATERİAL METHOD

In our study, 30 adult male Sprague Dawley rats were used with same biological and physiological characteristics, weighing between 250 and 300 g in Experimental Animal Research Unit of Trakya University. During the experimental period, all of our subjects were fed with daily drinking water and pellet feeds (Purina) containing 21% crude protein under optimal laboratory conditions (22 ± 1 ^{0}C, 12 hours light / dark cycle). The experiment were formed total of 3 groups.

Group I: (Control, n = 6): Citrate buffer (with a pH of 4.2; 0.1M) as a single dose administered intraperitoneally (ip).

Group II: (Diabetes, n = 12): Streptozotocin (dissolved in 0.1M citrate buffer with a pH of 4.2), given as a single dose of 40 mg / kg by the rope route.

Group III (A): (Diabetes + mild exercise, n = 12): STZ with diabetes (40 mg / kg single dose) starting 3 days ago, 5 days a week until the end of the experiment at a low speed exercise (10 m / min speed 30 min) applied to the group.

STZ (Sigma Aldrich Chemicals, USA) was used to generate chemically experimental diabetes. Twenty-four male rats that were diabetic and diabetic + mild exercise group were injected into a single dose of 40 mg / kg ip of STZ dissolved in citrate buffer. After the two days end of STZ injection, blood glucose levels were measured with blood samples taken from the tail vein by glucometer.

Body weights of all subjects were measured at the beginning and end of the experiment. Also both testis weights were measuredend of the experiment. Four weeks after diabetes and exercise process, testis of all subjects were excised in under anesthesia.

Exercise Procces

After 3 days, a single dose of 40 mg/kg STZ was given. For 4 weeks, mild exercise was performed and the experiment was terminated. A 30 min exercise program was applied to the diabetes +mild exercise group at a speed of 10 m/min for 5 days a week on the treadmill using a motorized running rope prepared for rats.

Light Microscopıc Examination

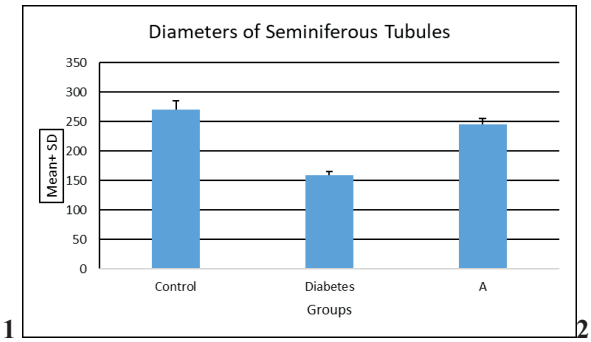
For light microscopic examinations, the testes were taken into paraffin blocks. 5 µm thick sections were taken from these blocks by using Leica RM-2245 cylinder microtome. The sections were stained with H&E (Hematoxylin + Eozin) in order to reveal the histological structure changes of the testis.

With the use of the same preparations, seminiferous tubule diameters of testis biopsy specimens of all subjects were measured using an ocular micrometer at x20 magnification and these measurements were performed on cross section of 10 randomly selected 10 tubules in round or round testis sections taken from each animal [9].

RESULTS

Blood Glucose Levels

Blood glucose levels of all experimental were measured by glucometer before and after STZ administration control diabetes and exercise groups. The blood glucose levels of diabetes groups and diabetes+ mild execise groups at the beginning of the experiment (2 days after STZ administration) were significantly $p < 0.0001$ and $p < 0.001$.



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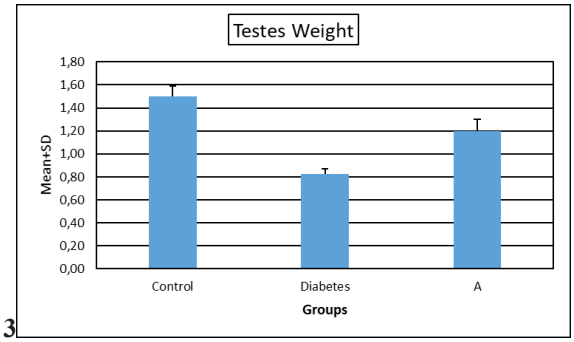

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Table 1. Blood glucose levels of control, diabetes and exercise groups.

A: Mild Exercise AL: After diabetes level B: Before diabetes level

*p<0.0001 compared with the control group, ** p <0.001 diabetes group compared to the statistically significant difference was determined.

Table 2. Seminiferous Tubules Diameters belonging to control, diabetes and exercise groups.

A: Mild Exercise

*p<0.0001 compared with the control group, **p <0.001 diabetes group compared to the statistically significant difference was determined.

Table 4. Testes weights of control, diabetes and mild-exercise groups.

A: Mild Exercise, **p<0.001 compared with the control group, ***p <0.01 diabetes group compared to the statistically significant difference was determined.

Diameters of Seminiferous Tubules

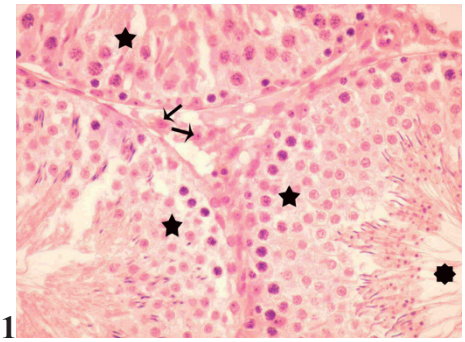
Changes in seminiferous tubules due to diabetes are shown by measuring tubular diameter. When compared with the control group of diabetes and mild exercise group, seminiferous tubule diameters of diabetes group have showed statistically significant decrease at p<0.001 and p<0.01 levels compared to the mild exercise.

Testes Weights

When all experimental groups were compared with control group, it was observed that decreased significantly testis weights. This decrease was statistically significant in the diabetes group p <0.001, diabetes group compared to mild exercise group p<0.01 level was found to be statistically significant.

Histological Results

Light microscopic results of the control group: When the testicular tissue sections of the taken from the subjects of the control group and stained with H & E were examined, the seminiferous tubules were generally of regular structure and were found to be smooth in diameter and size.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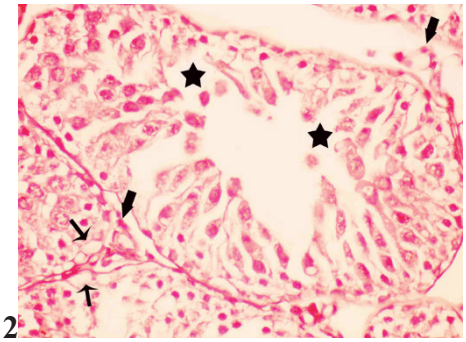
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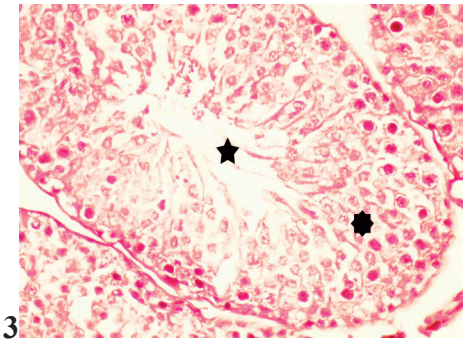
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Figüre 1. H & E staining in the testis section belonging to the control group. Seminiferous tubules ( ) borders and germ cells (★). The oval-shaped nucleus of the polygonal Leydig cells ( ) 400.

Figüre 2. H&E staining in testis section of diabetes group: In seminiferous tubules, irregularity and spermatogenic cell lineage are disrupted, germinal series cells are lost (★),separated from basal membranes and vacuoles ( ). Irregular Leydig cells ( ).400.

Figüre 3. H&E staining of the testis section of the mild exercise group. Uniform seminiferous tubules (★),spermatogenic cell losses between the extensions of Sertoli cells ( ).400.

Light microscopic findings of diabetes group: In the testicular tissue of the diabetes group, atrophic changes were observed in most seminiferous tubules. In addition to the reduction in the size of these atrophic tubules, it was striking that the normal structure was disrupted and the cells were separated from the basement membrane and discharged into the lumen. The normal polygonal structures of Leydig cells in the interstitial area were disrupted and their core structures were irregular as well as cytoplasmic losses.

Light microscopic findings of mild exercise group: When H&E stained testicular tissue sections were examined, regular placement between Sertoli and germ cells and intact-cell connections were observed. It

was observed that the cell loss in the epithelium of the seminiferous tubules decreased, and the number of seminiferous tubular degeneration and atrophic tubules decreased significantly. Besides There was determine an increase in the number of spermatogenic cells. In interstitial connective tissue, more smooth-looking Leydig cells were observed around the capillary vessels.

DİSCUSSİON

Diabetes mellitus is a metabolic disease that is common in the world and affects many organs in the early and late period. Diabetes is known to cause harm to all organs of the body in primary or secondary periods. Enzlin et al. [10] and Beshay et al. [11] have showed that in studies diabetes causes reproductive disorders in both male and female individuals STZ, one of the substances used in the formation of experimental diabetes, it is demonstrates by destroying β cells in the pancreas diabetogenic effect. Studies have reported that insulin-dependent diabetes in adult rats can be generated by injection of 40-60 mg/kg of STZ, usually a single dose of intravenous or at least 40 mg / kg ip [12,13]. Exercise has been considered as one of the three main components of diabetes treatment since many years, in addition to diet regimens and medicines. Emphasizing the role of preventing the development of possible type II diabetes. It is stated that patients with Type II diabetes should be encouraged to increase their physical activity. Furthermore, it is important to adopt regular exercise as a way of life. Regular exercise reduces blood glucose levels, which is very important in controlling diabetes. In the last few years it has been suggested that exercise reduces the risk of development of various diseases. However, it has been reported that heavy exercise and training impair male reproductive capacity and testicular structure, and that regular exercise has an important role in reducing type 2 diabetes, cardiovascular diseases and some types of cancer [14]. It is known that diabetic men have some changes in testosterone production and spermatogenesis. It has been reported that a person with diabetes who exercise regularly maintains some changes due to diabetes in the testis tissue. In this study, while seminiferous tubules of rats belonging to the control group were observed as normal and healthy, in the diabetes group was found histopathological changes. Distortions in the structure of the seminiferous tubules, separation of the germinative epithelium from connective tissue, invagination in the tubule wall, separation of the seminiferous tubule epithelial cells from the primary spermatocytes were seen. These microscopic findings support the study of Oksanen [15].

CONCLUSİON

In our study; diabetes also causes damage to the testis tissue as in many tissues. In order to prevent these damages, exercise practice has become very popular nowadays. Thus, it was consensus that the negative effect of diabetes on testis will be corrected with mild exercise.

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OP 007

Comparison of protective effects of betamethasone, dexamethasone and methylprednisolone on experimental ischemia/reperfusion injury of rat ovary

Sakin O, Angin A.D. , Koyuncu K., Akalın E.E., Basak K., Kaptanagasi A.O., Cikman M.S.

Introduction

Ovarian torsion is one the rare and reversible reason of the ovarian damage which usually could not be reversed (1). Ovarian torsion should be treated with surgery as soon as possible to preserve fertility (2). It is believed that the damage is caused by the inflammatory cytokines, lipid peroxide levels and ischemia- modified albumin which are increased in the tissue due to the decreased blood flow. On the other hand detorsion could also cause damage in the ovary because of the reactive oxygen species increase in the tissue.

Corticosteroids have been used in treatment of the damage caused by the inflammation process in the different areas (3). High dose methylprednisolone is shown to reduce the ischemia/ reperfusion damage of rat ovary (4).

The aim of the study is to compare the effects of betamethasone, dexamethasone and methylprednisolone in ischemia/reperfusion injury in an experimental rat adnexal torsion model.

Material and Methods

For this study, 40 female Wistar rats were used, and With the exception of the normal group, an ovarian torsion procedure was implemented in all other groups for 3 hours. Then, a detorsion procedure was implemented to the groups for 3 hours. Medications were given intraperitoneally, 30 minutes before the detorsion procedure. Finally, 1 ml of blood samples was drawn for anti mullerian hormone examination, while the ovaries which were torsioned for the histological examination were extracted from all rats.

Rat model

A total of 40 female adult Sprague-Dawley rats weighing between 190–260 g were used in this study. The animals were obtained from the University of the Medical Sciences Experimental Animal Laboratory. Animals were housed at the Animal Research Center of University of Medical sciences and were kept under constant laboratory conditions at room temperatures of 20 °C to 22 °C in a 12-hour light-dark cycle and were allowed free access to food and water. The surgical procedures described below were performed in accordance with the guidelines of the National Institutes of Health. The study team was blinded to the randomized groups. The rats were separated randomly into five groups consisting of eight rats each:

normal group; no intervention was made for this group,
torsion-detorsion group were subjected to left adnexal torsion for two hours and received no treatment,
torsion-detorsion + betamethasone group had two hour torsion and at the end treated with 3 mg/kg betamethasone intraperitoneally,
torsion-detorsion + dexamethasone had two hour torsion and at the end treated with 4 mg/kg betamethasone intraperitoneally,
torsion-detorsion + methylprednisolone had two hour torsion and at the end treated with 10 mg/kg methylprednisolone intraperitoneally.

All rats were weighed and anesthetized with a mixture of 50 mg/kg body weight ketamine hydrochloride (Ketalar, Eczacıbaşı, Istanbul, Turkey) and 10 mg/kg body weight xylazine (Rompun, Bayer, Leverkusen, Germany) injected intraperitoneally. With a longitudinal incision in the midline area of the lower abdomen, a small peritoneal incision was made, and the uterine horns and adnexa were located. In the ischemia groups , left unilateral ovarian ischemia was induced by applying two vascular clips using a silicone catheter to increase the pressure, one below the left ovary and the other in the uterus. In the torsion group, the clips were removed after a 2-hour period of ischemia, and the left ovary was surgically removed for histological examination. At the end of 24 hours, rats were anesthetized by intraperitoneal administration of 50 mg/kg ketamine hydrochloride and 10 mg/kg xylazine hydrochloride, and relaparatomy was done in all groups of rats. A 1.5–2 ml blood sample was taken from the abdominal aorta of each rat for biochemical examination. After taking the blood samples, the left ovaries were surgically removed. Ovaries were bisected to determine histopathological damage in ovarian tissue. All rats were sacrificed by drawing arterial blood from the aorta of the abdomen.

Ovarian tissues were fixed in 10 % neutral buffered formalin solution for 48 h, dehydrated, cleared inxylene, and embedded in paraffin. Sections (5 µm) were cut and stained with hematoxylin and eosin (H &E) and observed by light microscopy to investigate histological alterations. The criteria for ovarian injury were: follicular cell degeneration, vascular congestion, hemorrhage and infiltration by inflammatory cells. The ovarian sections were all analyzed by the same histologist blinded to the groups (K.B).

Blood samples were withdrawn from the ascending aorta of rats, placed into plain tubes containing separation gels and allowed to clot for 30 min. Serum samples were prepared by 15 min centrifugation at 3000 rpm. Tissue and blood samples were kept at −80 °C until biochemical analysis.

Results

According to the histopathological damage scores, the least damage was seen in the normal group and the most damage was seen in the torsion-detorsion group. The methylprednisolone treatment seems to be protective for the damage in terms of vascular congestion (p= 0.194), inflammation (p= 0.157), edema(p= 0.118) and cellular degeneration (p= 0.317) (Table 1). The dexamethasone and betamethasone treatment seems to be protective for tissue damage in inflammation (p= 0.317, 0,063), cellular degeneration (p= 0.083, 0.285) and edema (p= 0.212, 0,162). Primordial and primer folliculle counts were statistically different between normal and the torsion and steroid treatment group. On the other hand athresic folliculle counts were statistically different

between torsion and normal group while steroid treatment groups and normal group were not statistically different. Athresic follicle counts were statistically different between normal and torsion groups (p= 0.024). On the other hand methylprednisolone (p=0.083), dexamethasone (0.180) and betamethasone (0.999) group did not statistically differ from the normal group (Table 2). Besides AMH levels were statistically different between torsion and detorsion, whereas methylprednisolone, betamethasone and dexamethasone groups were similar to normal group.

Discussion

Several factors were suspected to be the reason of the ischemia/reperfusion injury (5). Glucocorticoids reduce phospholipase A and cyclo-oxygenase-2 expression, vascular permeability and edema (6). Evaluation of histological parameters in the present study also showed that treatment with methylprednisolone reduced the extent of ischemia/reperfusion tissue injury better than betamethasone and dexamethasone in rat ovary. Our findings show that all of the given glucorticoids protects ovarian follicle count against injury and AMH levels were similar to the normal group after treatment. In conclusion, methylprednisolone treatment seems to have a better choice of glucocorticoid therapy in rat ovary. However, further studies are needed to determine the best glucocorticoid therapy for ischemia/reperfusion injury of ovary.

Table 1. Comprison of histopathologic features between study groups

Steroidler	Normal	Torsion	P*** *	Detortion+ Dexamethasone	P*	Detortion+ Betametasone	P**	Detortion+ Methylprednisolone	P***
Edema									
Mean SD	1,33±0,52	2,50±0,55	**0,011**	1,83±0,75	0,212	2,00±,89	0,162	0,67±0,82	0,118
Median- IQR	1,00 (1,00-2,00)	2,50 (2,00-3,00)		2,0 (1,0-2,0)		2,0 (1,0-3,0)		0,5 (0,0-1,0)	
Vascular congestion									
Mean SD	0,83±0,98	1,33±1,03	0,403	2,17±0,75	**0,036**	3,00±0,00	**0,002**	1,50±0,84	0,238
Median- IQR	0,50 (0,00-2,00)	1,00 (1,00-2,00)		2,0 (2,0-3,0)		3,0 (3,0-3,0)		1,0 (1,0-2,0)	
İnflamation									
Mean SD	0,50±0,55	1,00±0,00	0,056	0,33±0,52	0,575	1,00±0,89	0,299	0,33±0,52	0,575
Median- IQR	0,50 (0,00-1,00)	1,00 (1,00-1,00)		0,0 (0,0-1,0)		1,0 (0,0-2,0)		0,0 (0,0-1,0)	
Cellular degeneration									
Mean SD	0,33±0,52	1,83±0,98	**0,011**	0,50±0,55	0,575	1,00±1,26	0,368	0,17±0,41	0,523
Median- IQR	0,00 (0,00-1,00)	1,50 (1,00-3,00)		0,5 (0,0-1,0)		0,5 (0,0-2,0)		0,0 (0,0-0,0)	
Hemorrrhage									
Mean SD	0,33±0,52	2,50±0,55	**0,003**	2,00±1,26	**0,028**	2,83±0,41	**0,002**	1,67±1,21	0,042
Median- IQR	0,00 (0,00-1,00)	2,50 (2,00-3,00)		2,5 (1,0-3,0)		3,0 (3,0-3,0)		1,5 (1,0-3,0)	

Table 2. Comparison of ovarian follicle counts and AMH levels between groups

Steroidler	Normal	Torsion	*P**	Detortion+ Dexamethasone	*P***	Detortion+ Betamethasone	*P****	Detortion+ Methylprednisolone	*P*****
Primordial follicle									
Mean SD	14,83±3,54	5,50±3,99	**0,010**	4,2±3,5	**0,004**	4,2±2,6	**0,004**	3,0±1,4	**0,004**
Median-IQR	15,50 (11,00-18,00)	4,50 (2,00-8,00)		3,0 (1,0-8,0)		4,00 (2,00-7,00)		3,0 (2,0-4,0)	
Primer follicle									
Mean SD	16,83±3,19	9,33±3,27	**0,010**	8,8±4,0	**0,006**	9,8±4,6	**0,010**	5,0±2,4	**0,003**
Median-IQR	18,00 (13,00-18,00)	9,00 (6,00-12,00)		9,5 (5,0-12,0)		9,5 (8,0-12,0)		4,0 (3,0-8,0)	
Secondary follicle									
Mean SD	7,83±3,92	9,17±2,93	0,466	5,8±3,7	0,569	5,2±2,6	0,196	3,5±1,6	**0,033**
Median-IQR	7,50 (4,00-12,00)	8,50 (8,00-12,00)		5,5 (4,0-7,0)		5,0 (,30-7,0)		3,5 (3,0-4,0)	
Tersier follicle									
Mean SD	4,17±1,33	4,67±2,16	0,739	5,5±3,1	0,616	5,5±2,1	0,211	5,0±2,4	0,513
Median-IQR	5,00 (3,00-5,00)	4,50 (3,00-6,00)		4,5 (3,0-7,0)		5,5 (4,0-6,0)		5,0 (3,0-7,0)	
Athresic follicle									
Mean SD	0,00±0,00	2,50±,55	**0,002**	0,5±0,5	0,056	1,0±1,7	0,140	0,2±0,4	0,317
Median-IQR	0,00 (,00-,00)	2,50 (2,00-3,00)		0,5 (0,0-1,0)		0,0 (0,0-2,0)		0,0 (0,0-0,0)	
AMH									
Mean SD	2,64±0,95	0,84±0,25	**0,004**	1,95±0,57	0,335	1,82±0,95	0,064	2,77±0,98	0,872
Median-IQR	2,59 (1,64-3,70)	0,92 (0,67-1,00)		1,85 (1,74-1,98)		1,39 (1,25-2,25)		2,98 (2,22-3,50)	

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PP 008

Overstress kills!...: Alterations of cumulus cell's DNA fragmentation and follicular fluid oxidative stress levels at patients with polycystic ovary syndrome

(Title of Abstract)

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Key Words: Cumulus cell, Follicular fluid, DNA damage, Polycystic ovary syndrome (PCOS), Oxidative stress

Abstract

It is known that bi-directional relationship between cumulus cells and oocyte has a significant importance on oocyte maturation. Follicular fluid secreted by granulosa cells provide the proper microenvironment which is essential for mature oocyte development.

This research is a prospective study. We exposed the relationship between DNA fragmentation of cumulus cells (with TUNEL positivity) and oxidative stress levels in follicular fluid of patients with PCOS (test) and male factor (control) group.
Patients with higher TOS levels had higher number of cumulus cells with DNA damage. Our results imply that correlation between DNA damage of cumulus cells and oxidative levels of follicular fluid may be interpreted with improper environment for oocyte maturation.

1. Introduction:

Under favour of all developments in intracytoplasmic sperm injection (ICSI) technique, a significant success has been gained. This improvement is accelerated by follicular monitoring, identification of top-quality embryos, embryo transfer and controlled ovarian hyperstimulation (COHS), procedures. Despite of all these progressions, still inefficiency at successful pregnancy outcome is seen at some of patients which had consulted assisted reproductive techniques (ART) [1]. Undoubtfully, picking up the best quality oocyte has a significant importance in this area [1, 2].
Polycystic ovary syndrome (PCOS) effects almost %5-10 of women at reproductive age. It can be said that it is a common and complex disorder which provides a poor oocyte quality [3]. Infertility is seen at %40 of patients who are diognosed with PCOS [3, 4]. As it is declared in literature parameters like oxidative stress, chronic inflammation, oocyte quality takes a significant importance in the success of ART techniques for PCOS patients [5, 6].
Oocytes which are released with ovulation are surrounded closely with cumulus cells. These cells provide the network

which supplies the proper microenvironment for oocyte. The connection between oocyte and cumulus cells is bidirectional and this connection ensures capable oocyte maturation [7].
Follicular fluid which is secreted by granulosa cells supplies the appropriate metabolites neccesary for oocyte maturation process. Because of that the properties of this fluid has a profound effect at follicular maturation process [3, 8]. Oocyte cumulus complex maturates in the environment provided by follicular fluid, and oxidative stres levels in this area has an impulse on oocte quality and clinic values like pregnancy outcomes, healthy placentation, implantation, embryological development [8].
It is known that apopitotic biomarkers of cumulus cells effects oocyte quality and clinic outcomes [9]. And apopitotic status of cumulus cells is commonly interpreted with oocyte maturation, fertilization, healthy pregnancy outcomes in previos studies.[9, 10].
It is aimed to investigate the relationship between follicular oxidative stress levels and apopitotic status of cumulus cells in PCOS patients and control group in this study. TUNEL procedure is followed to explore DNA damage of cumulus cells. Total anti-oxidant status (TAS) and Total oxidative status (TOS) is investigated with UV-visible spectrophotometry for oxidative stress in follicular fluid.

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2. Materials and Methods:

This was a prospective study investigating the relationship between DNA fragmentation of cumulus cells (with TUNEL positivity) and oxidative stress levels in follicular fluid of patients with PCOS and control group. The study was approved by the ethical committee of Gazi University Faculty of Medicine and 10 patients with PCOS (test) and 10 patients with male factor (control) without any documented female factor were recruited between March 2018 and December 2018. Two independent and blinded researchers had run experiments and analyzed the data. PCOS (test) and male factor (control) patients with consent were recruited. Cumulus cells were mechanically dissected, fixed on polylysine processing slides and pre-treated with paraformaldehyde for TUNEL procedure. Follicular fluids (without flushing) were collected during oocyte pick-up, centrifuged and subjected to total antioxidant status (TAS) and total oxidative stress (TOS) assays using UV-visible spectrophotometry.

3. Results and Discussion:

TAS levels of follicular fluid were not found significantly different between control and PCOS group. However, TOS results of follicular fluid belonging to PCOS patients were significantly higher than control group ($p<0.002$). TUNEL positive cumulus cells were found to be higher for PCOS patients. In addition, PCOS patients with high TOS levels in follicular fluid had higher numbers of cumulus cells with DNA fragmentation.

4. Conclusion:

Number of TUNEL positive cumulus cells in PCOS patients were higher than control group. In this group, TOS levels of follicular fluid were also high.

DNA fragmentation of cumulıs cells were found correlated with TOS levels

This correlation was interpreted with improper environment for oocyte maturation.

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PP 009

THE EFFECT OF HYALURANIC ACID SPERM SELECTION ON INTRACYTOPLASMIC SPERM INJECTION OUTCOME OF PATIENTS WITH OLIGOZOOSPERMIA

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ABSTRACT

OBJECTIVE: To evaluate the efficacy of hyaluronic acid binding (HA) sperm selection (PICSI) compared to conventional sperm selection (control) prior to ICSI in patients with severe or moderate oligozoospermia in terms of blastocyst development and clinical success rates.

METHODS: This is a retrospective data analysis. Women younger than 39 years old with normal ovarian reserve, men with severe or moderate oligozoospermia and cycles with blastocyst embryo transfer were included in the study. Fertilization, top and good quality (TQ-GQ) blastocyst development rate, ongoing pregnancy and miscarriage rates were compared between the groups.

FINDINGS and ARGUMENT: A total of 146 ICSI cycles were included in this study. Of those cycles 46 and 100 cycles were carried out with PICSI and conventional sperm selection, respectively. There was no significant difference between the groups regarding fertilization, TQ-GQ blastocyst development, ongoing pregnancy, and miscarriage rates after adjusting for number of retrieved oocytes and transferred embryos. Number of retrieved oocytes were the only significant factor effecting blastocyst development and ongoing pregnancy rate. HA-binding% were significantly correlated with sperm concentration but not with sperm motility.

CONCLUSION: The present data indicates that using HA-binding for sperm selection before ICSI does not have any additional benefit over conventional sperm selection in patients with oligozoospermia. In cycles with low sperm count number of oocytes is the most significant predictor of clinical success. As the number of retrieved oocytes increases TQ-GQ blastocyst development and ongoing pregnancy rate increase compensating for the low sperm count.

1. INTRODUCTION

The ultimate aim of IVF is to obtain a live birth. Despite the introduction of many new techniques, IVF success rates has remained unchanged in the past decade [1]. Embryo development and ICSI outcome is dependent on both the egg and sperm quality. Although research has focused mainly on women, there is an increasing interest on sperm selection techniques aiming this might improve the success rates if competent sperm can be selected for ICSI. In spermiogenesis, during sperm plasma membrane remodeling, along with the formation of zona pellucida, receptors for hyaluronic acid (HA) are also formed [2]. Selection of competent sperm based on its ability to bind to HA has been suggested as one of the methods to assess sperm quality by mimicking natural sperm selection which is called physiologic ICSI (PICSI) [3]. It has been reported that Hyaluronan-selected sperm have reduced levels of DNA damage and aneuploidy. [4, 5].

There are a number of studies which suggested a beneficial effect of HA sperm selection on assisted reproductive technology (ART) outcomes [5–7]. However, a recent randomized controlled trial (RCT) comparing PICSI and conventional ICSI showed no statistical difference in embryo quality and pregnancy rates [8].

These studies did not categorize sperm parameters and usually included males with normal sperm concentration and motility. There is only one retrospective study comparing ICSI and PICSI with male factor infertility indicating that cycles that used PICSI technique had a considerably higher chance to achieve pregnancy but the number of patients in this study is so small [9].

In the present study we aim to evaluate the efficacy of PICSI compared to conventional sperm selection prior to ICSI in patients with severe or moderate oligozoospermia in terms of blastocyst development and clinical success rates.

2. MATERIALS AND METHODS

This is a retrospective study including cycles carried out at Medipol University IVF Center between 2015 to 2017. Women younger than 39 years old with normal ovarian reserve, men able to produce freshly ejaculated sperm with severe or moderate oligozoospermia and cycles with blastocyst embryo transfer were included in the study. Moderate oligospermia and severe oligospermia is defined according to WHO 2010 criteria as sperm concentration of 5-10 million/ml and <5 million/ml, respectively. Primary outcome of the study was ongoing pregnancy rate. Secondary outcomes were fertilization, top and good quality (TQ-GQ) blastocyst development rate, and miscarriage rates. Sperm samples were collected on the day of egg collection (OPU). Sperm was kept at room temperature for 30 to 60 min to allow liquefaction of the sample. Semen analysis was performed according to WHO criteria. Gradient centrifugation was performed to separate the cellular components of the sperm. PICSI sterile petri box has been used for mature sperm selection (PICSI® Sperm Selector)

For statistical analysis; student's t-test, chi-square test, linear and binary logistic regression analysis were used where appropriate. Differences were considered significant when a P value was <05.

3. RESULTS AND DISCUSSION

A total of 146 ICSI cycles were included in this study. Of those cycles 46 and 100 cycles were carried out with PICSI and conventional sperm selection, respectively. In 5 cycles no embryo transfers were achieved due to ovarian hyper stimulation syndrome risk. Female age (29.2 ± 4.0 vs 29.7 ± 4.1), male age (33.4 ± 4.4 vs 33.2 ± 4.5), and sperm concentration (3.5± 3.8 vs 4.4 ± 3.5) were similar between the groups.

However, number of retrieved oocytes (16.3 ± 9.1 vs 13.1 ± 5.6) were significantly higher and number of embryos transferred were lower (1.6 ± 0.5 vs 1.9 ± 0.3) in PICSI compared to control group. Mean HA-binding in PICSI group was 18.3 ± 24.7%. There was no significant difference between the groups regarding fertilization (83.6±16.1 vs 83.9±14.1, p=0.91), TQ-GQ blastocyst development (49.3±22.2 vs 50.0±23.9, p=0.90), ongoing pregnancy (56.1% vs 43.1, p=0.16), and miscarriage rates (17.9 % vs 21.8%, p=0.67). Sperm selection with PICSI or conventional method did not affect fertilization, TQ-GQ blastocyst development, ongoing pregnancy and miscarriage rates after adjusting for number of mature oocytes and transferred embryos. Number of retrieved oocytes were the only significant factor effecting blastocyst development and ongoing pregnancy rate.

HA-binding% were significantly correlated with sperm concentration (r=0.554; p<0.001) but not with sperm motility. When PICSI group were divided into subcategories according to HA-binding (<20% vs >20%), there were no significant differences in success rates between <20% or >20% HA-binding groups.

Table 1: Comparison of PICSI and ICSI groups

	PICSI (n=46)	ICSI (n=100)	P value
Female age	29.2 ± 4.0	29.7 ± 4.1	NS
Male age	33.4 ± 4.4	33.2 ± 4.5	NS
Sperm concentration (mean±SD)	3.5± 3.8	4.4 ± 3.5	NS
Retrieved oocytes (mean±SD)	16.3 ± 9.1	13.1 ± 5.6	0.009
Transferred embryos (mean±SD)	1.6 ± 0.5	1.9 ± 0.3	0.01
Fertilization rate (%)	83.6±16.1	83.9±14.1	0.91
Blastocyst development rate (%)	49.3±22.2	50.0±23.9	0.90
Ongoing pregnancy rate (%)	56.1	21.8	0.16
Miscarriage rate (%)	17.9	21.8	0.67

4. CONCLUSION

The present data indicates that using HA-binding for sperm selection before ICSI does not have any additional benefit over conventional sperm selection in patients with oligozoospermia. In cycles with low sperm count increased number of oocytes is the most significant predictor of clinical success.

As the number of retrieved oocytes increases TQ-GQ blastocyst development and ongoing pregnancy rate increase compensating for the low sperm count.

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PP 010

THE ROLE OF ELEVATED BASAL FOLLICLE-STIMULATING HORMONE IN YOUNG INFERTILE WOMEN UNDERGOING GONADOTROPIN STIMULATION/ INTRAUTERINE INSEMINATION TREATMENT

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ABSTRACT

The objective of the study is to determine the role of elevated day 3 follicle stimulating hormone (FSH) in young women (<35 years) undergoing gonadotropin-stimulation/intrauterine insemination treatment. 329 women (<35 years) who underwent first cycle of gonadotropin-stimulation/intrauterine insemination treatment over 2 years were included in this retrospective study. Those were divided into two groups according to their day 3 FSH levels. Women with FSH level ≥10 U/L was recorded as elevated FSH group, while women with FSH level <10 U/L was as normal FSH group. 30 (9.1%) women were in elevated FSH group while others (90.9%) were in normal FSH group. After the treatment, 38 (11.6%) women had clinical pregnancy. Six of these were in elevated group and 32 of them were in normal group. Clinical pregnancy rate was significantly lower in elevated FSH group than in normal FSH group (5.0% vs 10.4%, respectively). More gonadotropin doses were used in elevated FSH group (815.1±370.1 vs 645.7±242.5 IU, respectively; p<0.018). In conclusion, women younger than 35 years with elevated day 3 FSH level had lower clinical pregnancy rate after gonadotropin stimulation/intrauterine insemination treatment compared to women with normal FSH level and needed to use higher gonadotropin doses to achieve pregnancy.

Keywords: follicle stimulating hormone; gonadotropin stimulation; intrauterine insemination

1. INTRODUCTION

Elevated basal follicle stimulating hormone (FSH) levels are associated with poor reproductive outcomes, especially in older women [1,2]. On the other hand, there is no clear view of how such a condition exists in young women at the early stages of fertility treatments [3,4]. Therefore, we aimed to determine the role of elevated day 3 FSH in young women (<35 years) undergoing gonadotropin-stimulation/intrauterine insemination treatment.

2. MATERIALS AND METHODS

This retrospective study included 329 young women (<35 years) who underwent first cycle of gonadotropin-stimulation/intrauterine insemination treatment over 2 years at infertility department of our hospital. Those were divided into two groups according to their day 3 FSH levels. Women with FSH level measured ≥10 U/L was recorded as elevated FSH group, while women with FSH level <10 U/L was as normal FSH group. The study was approved by the Institutional Review Board of the hospital. Demographics (woman's age, body mass index, smoking status, infertility duration, sperm parameters), clinical data (gonadotropin dose, treat-

ment duration, peak estradiol, number of preovulatory follicles, endometrial thickness) and clinical pregnancy rates were compared between the groups. Clinical pregnancy was defined as the detection of an intrauterine gestational sac by transvaginal ultrasonography.

3. RESULTS AND DISCUSSION

Of 329 cycles, 30 (9.1%) were from women with a basal FSH of 10 U/L or greater, while FSH levels for the others (90.9%) were in normal range. After the treatment, 38 (11.6%) women had clinical pregnancy. Six of these pregnancies were in the elevated FSH group and 32 of them were in the normal FSH group. Clinical pregnancy rate was significantly lower in the elevated FSH group than in the normal FSH group (5.0% vs 10.4%, respectively; p<0.001). More gonadotropin doses were used in elevated FSH group than in the normal FSH group (815.1±370.1 vs 645.7±242.5 IU, respectively; p=0.018). The other demographic and clinical parameters were statistically similar between the groups (Table 1).

Table 1. Characteristics of the groups

	Elevated FSH Group (N=30)	Normal FSH Group (N=299)	p
Woman's age (year)	28.9±2.5	29.1±2.4	NS
BMI (kg/m^2)	24.4±3.4	25.2±3.1	NS
Smoking (n)	3 (10.0)	33 (11.0)	NS
Infertility duration (years)	2.0±0.2	2.0±0.4	NS
Basal normal sperm morphology (%)	6.8±1.2	6.9±1.6	NS
TMSC (x10^6/ml)	55.1±24.2	56.1±24.4	NS
Gonadotropin dose (IU)	815.1±370.1	645.7±242.5	0.018
Induction duration (day)	8.9±2.4	8.8±2.2	NS
Peak E2 (day prior to hCG) (pg/ml)	480.1±184.2	460.4±164.3	NS
No. of preovulatory follicles (≥18mm)	1.2±0.4	1.4±0.3	NS
Endometrial thickness on hCG trigger day (mm)	9.0±1.2	8.8±1.1	NS
Clinical pregnancy (n)	6 (5.0)	32 (10.4)	<0.001
BMI: Body mass index; TMSC: total motile sperm count; E2:Estradiol NS: Not significant; p < 0.05 is considered statistically significant			

Although many studies have reported that basal FSH has clinical importance in predicting IVF results, there are not enough studies associated with gonadotropin-stimulation/intrauterine insemination treatment results. In their study conducted on subfertil ovulatory women, Steeg et al. found a decrease in spontaneous pregnancy rate when basal FSH value was above 8 U/L [5]. On the other hand, in another study, Souter et al. reported that increased FSH levels (≥10 U/L) in gonadotropin-stimulation/intrauterine insemination cycles of young women had similar results with normal FSH levels after higher dose of gonadotropin treatment [6]. While our findings support Steeg's study in terms of pregnancy outcomes, they also support Souter's study regarding the treatment modality.

4. CONCLUSION

Women younger than 35 years with an elevated day 3 FSH levels had lower clinical pregnancy rate after gonadotropin stimulation/intrauterine insemination treatment compared to women with normal FSH levels. And in women with elevated day 3 FSH levels, it is necessary to use higher gonadotropin doses to achieve pregnancy.

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PP 011

COMPARISON OF TIME-LAPSE MORPHOKINETIC PARAMETERS BETWEEN GOOD QUALITY EMBRYOS WITH KNOWN LIVE BIRTH DATA AND THOSE FAILED TO ACHIEVE PREGNANCY.

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Objective:

Embryo selection is one of the most delicate steps in assisted reproduction and embryo transfer cycles. Many embryo selection options are currently available, however no invasive or non-invasive method have proven its superiority according to literature. Time-lapse morphology (TLM) allows us to screen morpho-kinetic behaviour of each embryo from zygote to blastocyst stage by acquiring sequential images. Due to its non-invasive nature and promise to maintain an uninterrupted culture environment, TLM has been widely accepted. Besides, the effort to implement an optimal algorithm for embryo selection is still going on with the data collected retrospectively from embryos with known implantation or live birth outcome. With that data accumulation, randomized controlled trials may only become possible to conduct, in order to see if the algorithms from those variable data are suitable to predict successful embryo implantation. This study aims to compare the morphokinetic differences between embryos with live birth outcome and those who failed to implant to achieve a pregnancy despite good embryo morphology. TLM values of embryos who caused pregnancies ended with miscarriage were also considered. Variables of age, endometrial thickness, and day of transfer were also investigated.

Materials and Methods:

This retrospective analysis included a total of 70 intracytoplasmic sperm injection (ICSI) cycles performed between August 2015 and May 2017 with a total of 82 embryos selected for single embryo transfer who i) resulted in live birth (Group 1, n=27), ii) negative pregnancy (group 2, n=45) and iii)early pregnancy lost (within first 13 weeks of pregnancy, Group 3, n=10). Microinjected oocytes were immediately transferred onto Primo Vision culture dishes in pre-equilibrated embryo culture medium (G-TL, Vitrolife, Goteborg, Sweden) in incubators with oxygen control (Panasonic, MCO-5AC, Japan) and implemented with Primo Vision EVO+ microscopes. Time lapse system is set in order to acquire images in every 7 min. Timings of second polar body exclusion (PB2E), pronuclei appearance (PNA), PN centralization (PNC), PN fading (PNF), cleavage to two, three, four and five cells (t2, t3, t4, t5, respectively), fully compaction (FC), blastulation (BL) and blastocyst expansion (BE) were recorded from PrimoVision Time-Lapse System (Vitrolife, Goteborg, Sweden) retrospectively. Also time differences between PNA and PB2E, PNC and PNA, PNF and PNA, cell cycle two (cc2=t3-t2) and cell cycle three (cc3=t5-t4) were calculated. Comparison of timings in three groups was calculated using One way ANOVA test in SPSS and results are displayed as minutes ± standard deviation.

Results:
Mean age and endometrial thickness values were comparable in all groups. Mean timing of PB2E, PNA, PNC, PNF, t2, t3, t4, t5, FC, BL and BE, as well as calculated differences for all groups were compared. There was no statistically significant difference between any parameters and any checkpoint gaps obtained in all groups (Table 1).

Conclusions:
In our study no morpho-kinetic differences were found between good quality embryos who caused live birth, negative pregnancy or miscarriage. It can be speculated that among good quality embryos, when multinucleation and asynchronized cleavage are eliminated, embryo dynamics using TLM cannot make a prediction for successful implantation and live birth. The age, endometrial thickness and the day of transfer do not correlate with successful prediction. More retrospective data should be conducted with proven outcome of cultured embryos in order to build predictive algorithms.

Table 1. The comparison of time-lapse morpho-kinetic parameters between the study groups.

	Live birth N=27	No pregnancy N=45	Miscarriage N=10
PB2E, h	3.59±1.56	3.27±1.50	2.53±1.03
PNA, h	9.58±2.62	9.95±2.75	9.48±1.63
PNC, h	17.30±4.27	16.10±5.30	18.18±4.04
PNF, h	23.25±2.51	23.93±3.08	24.72±3.51
PNF-PNA, h	14.06±3.89	13.98±3.39	15.24±3.44
PNA-PBE2E, h	6.48±3.30	7.01±2.69	7.46±2.14
PNC-PNA, h	8.78±3.67	7.70±4.44	6.63±7.16
T2, h	25.99±2.64	26.51±3.43	27.66±3.42
T3, h	34.70±5.01	36.47±3.73	34.05±3.53
T4, h	37.34±5.08	37.95±3.74	36.50±2.29
T5, h	47.09±8.92	50.05±5.38	44.16±7.16
Cc2, h	8.71±4.06	9.95±3.56	6.39±4.66
Cc3, h	10.22±5.32	12.06±3.67	8.51±4.89
FC, h	83.29±12.83	85.45±11.48	90.66±5.11
BL, h	92.31±14.18	99.97±6.81	99.3
BE, h	100.81±14.36	105.63±7.65	108.75

Table 1

		Mean±SD			Mean±SD
PB2E	Group 1	215.13±93.48	**t3**	Group 1	2082.11±300.34
	Group 2	196.15±89.54		Group 2	2188.20±223.70
	Group 3	151.75±61.79		Group 3	2043.00±211.94
PNA	Group 1	574.58±157.19	**t4**	Group 1	2240.74±304.80
	Group 2	597.25±165.14		Group 2	2277.25±224.22
	Group 3	568.70±97.65		Group 3	2189.80±137.48
PNC	Group 1	1037.83±256.25	**t5**	Group 1	2825.35±535.46
	Group 2	966.00±317.95		Group 2	3003.00±323.03
	Group 3	1091.00±242.86		Group 3	2649.55±429.09
PNF	Group 1	1395.12±150.88	**cc2**	Group 1	522.48±243.71
	Group 2	1436.03±184.62		Group 2	596.90±213.72
	Group 3	1483.20±210.48		Group 3	383.60±279.82
PNF-PNA	Group 1	843.52±233.90	**cc3**	Group 1	613.32±319.20
	Group 2	838.78±203.30		Group 2	723.66±220.17
	Group 3	914.50±206.58		Group 3	510.50±293.53
PNA-PB2E	Group 1	389.21±198.21	**FC**	Group 1	4997.36±769.78
	Group 2	420.39±161.85		Group 2	5127.33±689.21
	Group 3	447.30±128.16		Group 3	5440.00±306.88
PNC-PNA	Group 1	526.96±220.47	**BL**	Group 1	5538.73±851.06
	Group 2	462.06±259.81		Group 2	5998.25±408.78
	Group 3	397.88±429.76		Group 3	595,00
t2	Group 1	1559.63±158.84	**BE**	Group 1	6048.55±861.74
	Group 2	1590.93±205.78		Group 2	6337.92±459.41
	Group 3	1659.40±205.29		Group 3	6525.00

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PP 012

DOES MALE AGE ALONE ADVERSLY AFFECT PREGNANCY RATE IN INTRAUTERINE INSEMINATION CYCLES?

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ABSTRACT

The objective of the study is to analyze the effect of male age on pregnancy rates in couples undergoing intrauterine insemination treatment. We retrospectively analyzed 376 intrauterine insemination cycles applied to women under 40 years of age in our Infertility Department during two year-period. The main outcome was clinical pregnancy. Of these, 35 (9.2%) were resullted in clinical pregnancy while the remaining ones (90.8%) had no clinical pregnancy. The mean male and female ages were both significantly younger in pregnancy cycles than in nonpregnancy cycles. No relationship between male age and pregnancy outcomes was detected after adjusting for confounding factors and the female age was the only factor affecting the occurance of clinical pregnancy. And, we have concluded that male age does not adversely affect pregnancy rates after intrauterine insemination treatment.

Keywords: clinical pregnancy; intrauterine insemination; male age

1. INTRODUCTION

The female age is an important factor influencing the likelihood of pregnancy in natural or conventionally treated cycles. Namely, pregnancy rate is known to decrease with advancing female age, however the effect of advancing male age on pregnancy outcome is less clear [1,2]. We therefore aimed to analyze the effect of male age on pregnancy rates in couples undergoing intrauterine insemination treatment.

2. MATERIALS AND METHODS

This retrospective study included infertile couples who underwent intrauterine insemination treatment during two year-period at Infertility Department of our hospital. The study protocol was approved by Institutional Review Board. Baseline demographic and clinical data, including female and male ages, sperm parameters and the ovulation induction agent used were collected. Women ≥ 40 years were excluded because of the low pregnancy rate expected in that age group The main outcome was clinical pregnancy, defined as the detection of an intrauterine gestational sac by transvaginal ultrasonography.

3. RESULTS AND DISCUSSION

The study population comprised 376 intrauterine insemination cycles. Of these, 35 (9.2%) were resullted in clinical pregnancy while the remaining ones (90.8%) had no clinical pregnancy. The mean male and female ages were both significantly younger in pregnancy cycles than in nonpregnancy cycles (33.4 vs 36.2 years,

p<0.001, and 28.6 vs 30.5 years, p =0.022, respectively). Sperm parameters and ovulation induction agent were comparable among the groups (Table 1).

The results of previous studies regarding the association between male age and pregnancy rate have been controversial. Belloc et al. reported a significantly decreased pregnancy rate in men older than 45 years old [3]. But, Tatsumi et al. found no relationship between male age and pregnancy rates [4]. In our study, no relationship between male age and pregnancy outcomes was detected after adjusting for confounding factors and the female age was the only factor affecting the occurance of clinical pregnancy (Table 2). Our findings support the documented negative impact of female age on infertility treatment [5,6].

Table 1. Characteristics of the groups

	Pregnancy Cycles (N=35)	Nonpregnancy cycles (N=341)	p
Male age (years)	33.4±4.3	36.2±8.2	<0.001
Female age (years)	28.6±4.1	30.5±6.1	0.022
Basal normal sperm morphology (%)	6.9±1.6	6.8±1.7	0.707
TMSC (x10^6/ml)	55.1±23.1	56.4±25.4	0.496
Ovulation induction agent			0.915
Clomiphene citrate	17 (48.6)	168 (49.3)	
Gonadotropin	18 (51.4)	173 (50.7)	
TMSC: total motile sperm count p < 0.05 is considered statistically significant			

Table 2. Adjusted odds ratios including confounding factors for clinical pregnancy

	Adjusted OR (%95 CI)	p
Male age (years)		
<35	Reference	---
35-39	1.42 (0.32-2.48)	0.253
40-45	0.88 (0.46-1.28)	0.215
>45	0.71 (0.41-1.98)	0.119

Female age (years) <35 35-37 38-39	 Reference 1.15 (0.74-1.78) 0.53 (0.34-0.89)	 --- 0.456 0.028
OR: Odds ratio; CI: Confidence interval p < 0.05 is considered statistically significant		

4. CONCLUSION

Male age alone does not affect pregnancy rates in infertile couples undergoing intrauterine insemination teratment. Therefore, intrauterine insemination can be recommended to infertile couples including advanced-age male partner.

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