5. Klinik Embriyoloji Derneği Kongresi

20-22 Eylül 2019

Radisson Blue Resort & SPA
Çeşme İZMİR

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Değerli Katılımcılar,

Klinik Embriyoloji Derneği Yönetim Kurulu olarak 20-22 Eylül 2019 tarihleri arasında Çeşme, İzmir'de düzenleyeceğimiz "5. Klinik Embriyoloji Derneği Kongresi"ni sizlere duyurmaktan mutluluk duyuyoruz.

Bu toplantıda tüp bebek uygulamalarında son gelişmeler, tartışmalı konular ve ART sonuçlarının laboratuvar perspektifinden nasıl maksimize edileceğine odaklanacağız. Konular, diğer toplantılarımızda olduğu gibi konularında önder, fikir lideri uluslararası davetli konuşmacılar tarafınca tartışılacaktır.

Bilimsel programın ana dili İngilizcedir. Simultane çeviri yapılacaktır.

Toplantının katılımcı hedef kitlesi üreme tıbbı/tüp bebek ile uğraşan tüm embriyologlar, biyologlar, kadın hastalıkları ve doğum hekimleri, androloji/infertilite ile uğraşan ürologlar ve yardımcı sağlık personelidir.

Bu toplantıda sizleri de aramızda görmekten kıvanç duyacağız.

Saygılarımla,

Kongre Başkanı

Prof. Dr. Lale Karakoç Sökmensüer

Lale Karakoç Sökmensüer

Thomas Ebner

Borut Kovacıc

Denny Sakkas

Necati Fındıklı

Evrim Ünsal

Engin Enginsu

Başak Balaban

Sinan Özkavukçu

Elif Ergin

1. GÜN 20 Eylül 2019 • Cuma		
14:50	**Kongre Açılış Konuşması**	*Lale Karakoç Sökmensüer* *(KED Başkanı)*
15:00-15.30	**2019 yılı itibariyle IVF tedavilerinde güncel durum: Ulusal kayıtlar ne söylüyor?**	***Oturum Başkanları:*** *Ahmet Zeki Işık, Timur Gürgan* ***Konuşmacı:*** *SK. Sunkara (UK)*
15.30-16.30	**I. OTURUM:** IVF Laboratuvarlarındaki güncel dinamikler	***Oturum Başkanları:*** *Ahmet Zeki Işık, Timur Gürgan*
15.30–15.50	Anahtar performans göstergelerinin (APG, key performance indicators, KPI) klinik ve laboratuvar yönetimine yansımaları	*A. Campell (İngiltere)*
15.50–16.10	Laboratuvarda iş akışı nasıl iyileştirilir?	*B. Kovacic (Slovenya)*
16.10 - 16.30	Laboratuvar parametreleri tek embriyo transferi uygulamalarında klinik karar süreçlerine nasıl entegre edilir?	*SK. Sunkara (İngiltere)*
16.30-17.00	**Kahve arası**	

2. GÜN 21 Eylül 2019 • Cumartesi		
	Güncel embriyo kültür sistemleri: "Doğa"'ya ne denli yakınız?	***Oturum Başkanı:*** *Tülay İrez* ***Konuşmacı:*** *D. Sakkas (ABD)*
10:30-11:30	**Cook Medikal Uydu Sempozyumu** ''Mineral yağ toksisitesi: Embriyo kültüründe kritik bir bileşen''	*Gloria Calderón*
11:30-12:00	**Kahve arası**	
12:00-13:00	**II. Oturum:** Başarılı IVF sonuçlarına klinik embriyolojinin etkisi	***Oturum Başkanları:*** *Necati Fındıklı, Aycan Işıklar*
12:00-12:20	Bir ampulü değiştirmek için kaç embriyolog gerekir?	*A. Campell (İngiltere)*
12:20-12:40	İleri sperm analizleri ve seçim araçları: Güncel durum	*D. Sakkas (ABD)*
12:40-13:00	Öploidi sonuçlarını en yüksek seviyeye çıkarmak için IVF laboratuvarının nasıl olması gerekir?	*D. Cimadomo (İtalya)*
13:00-14:00	**Öğle yemeği**	
14:00-15:00	Vitrolife Uydu Sempozyumu	
14:00-14:25	Time-Lapse Yapay Zeka algoritmalarıyla embriyo seçiminin geleceği	*Niels Ramsing*
14:25-14:50	Embriyo seçiminde anöploidi için preimplantasyon genetik testi	*Kathryn Gebhardt*
14:50-15:00	Tartışma	
15:00-15:30	**Kahve arası**	
15:30-16:30	**III. OTURUM:** Panel Oturumu: Güncel embriyo seçim yaklaşımları: Şimdiye kadar ne öğrendik?	***Oturum Başkanları:*** *Kerem Dirican, Cem Korkmaz*
15:30-15:50	Zaman aralıklı görüntüleme (time-lapse) ile seçim en iyisidir	*T. Ebner (Avusturya)*
15:50-16:10	Konvansiyonel seçim en üstünüdür	*G. Smith (ABD)*
16:10-16.30	Tartışma	
16:30-17:00	**Kahve arası**	
17:00-19:00	Klinik Embriyoloji Derneği Genel Kurulu	

3. GÜN 22 Eylül 2019 • Pazar		
10:00-11:00	**Sözlü Bildiri Oturumu**	***Oturum başkanları:*** *Gülçin Abban Mete,Sibel Bulgurcuoğlu Kuran*
11:00-11:30	**Kahve arası**	
11:30-13:00	***IV. OTURUM:*** **ÜYTE'de yeni laboratuvar teknolojileri**	***Oturum Başkanları:*** *Mehmet Cıncık, Berrin Avcı*
11:30-11:50	**IVF Laboratuvarında mikroakışkanlar: Güncel durum**	*G. Smith (ABD)*
11:50- 12:10	**Gamet ve embriyo kriyoprezervasyonu teknolojileri: Yeni bir şey var mı?**	*B. Kovacic (Slovenya)*
12.10-12.30	**Girişimsel-olmayan PGT-A: Hayal mi yoksa gerçek mi?**	*D. Cimadomo (İtalya)*
12:30	**Kapanış**	

Oral Presentations

AN UNUSUAL MULLERIAN ANOMALY IN AN INFERTIL PATIENT

Ayşe Köse[1], **Gülşah İlhan**[2]

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Aim: Congenital agenesis of the unilateral adnexia is a condition that has rarely been described in the literature. Mullerian duct anomalies are known causes of infertility and reproductive problems. The incidence of this abnormalities is not exactly known. The most widely accepted method of classification for a Mullerian duct anomaly is the ASRM classification. However, there are some rare anomalies inconsistent with the current classification.

Case: Herein, we report a rare case of Mullerian duct anomaly, unilateral ovarian and tubal absence with a normal uterus. The current case presents a 36-years-old female who was admitted to the In Vitro Fertilization Unit of Istanbul Research and Education Hospital with a complaint of primary infertility. The patient could not concieve within 2 years. Her gynecologic examination was uneventful except her left ovary could not visualized by transvaginal ultrasonography. Her hysterosalphingography revealed a normal uterus with unilateral (left) proximal tubal blockage (Figure 1). During diagnostic laparoscopy, a normal uterus was detected. Left adnexal agenesis was encountered. Both left ovary and left tuba could not detected. A streak ovarian tissue was encountered in the location of normal ovary (Figure2). Right tuba and ovary were normal and right tubal passage of methylene blue passage was positive. Her postoperative MRI revealed a normal urinary system (Figure 3). Her karyotype analysis was 46, XX.

Conclusion: The etiology of the adnexal anomaly remained unclear, although torsion or congenital defects were the most likely explanation. A rare case of Mullerian duct anomaly, unilateral ovarian and tubal absence with a normal uterus is presented to keep in mind that contralateral anomalies can lead to infertility.

Keywords: agenesis, hysterosalpingography, infertility, ovary, tuba

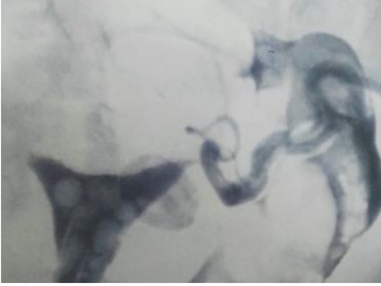

Figure 1

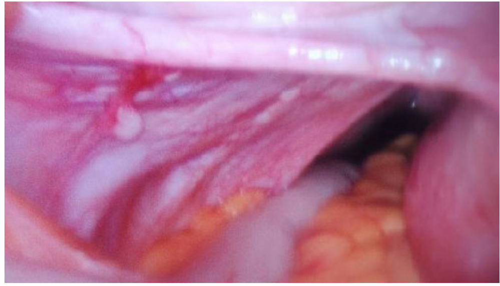

Figure 2

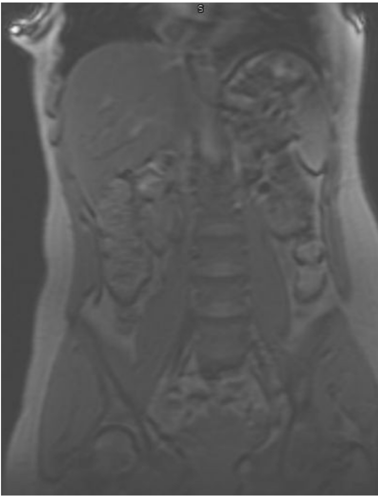

Figure 3

OVERSTRESS KILLS!...: ALTERATIONS OF CUMULUS CELL'S DNA FRAGMENTATION AND FOLLICULAR FLUID OXIDATIVE STRESS LEVELS AT PATIENTS WITH POLYCYSTIC OVARY SYNDROME

Duygu Dayanir[1], Halil Ruso[2], Ziya Kalem[3], Rabia Tural[4], Sanem Sarıbaş[5], Aylin Sepici Dinçel[6], Timur Gürgan[7], Candan Özoğul[8]

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[5]*Ahi Evran University School of Medicine, Department of Histology and Embryology*
[6]*Gazi University Faculty of Medicine, Department of Medical Biochemistry*
[7]*Bahcesehir University Faculty of Medicine, Department of Obstetrics and Gynecology*
[8]*University of Kyrenia Faculty of Medicine, Department of Histology and Embryology*

Objective: Study Question: Is there relationship between apopitosis levels of cumulus cells and oxidative stress in follicular fluid of patients with PCOS?

Study answer: Apopitosis of cumulus cells, oxidative stress levels in follicular fluid were found to be increased for Polycystic Ovary Syndrome (PCOS) patients. Also, apopitosis of cumulus cells were found to be correlated with total oxidative stress levels in follicular fluid.

Design: This was a prospective study investigating the relationship between apopitosis of cumulus cells and oxidative stress levels in follicular fluid of patients with PCOS with 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. Any further experiments were performed at Biochemistry and Histology Departments of Gazi University Faculty of Medicine. 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 oxidant status (TOS) assays using UV-visible spectrophotometry.

Results: TAS levels of follicular fluid were not found significantly different. However, TOS results of follicular fluid belonging to PCOS patients were significantly higher than control (male factor) group ($p<0.002$). Apopitotic cumulus cells were found to be higher for PCOS patients. Also, PCOS patients who had higher TOS levels at follicular fluid had more apopitotic cumulus cells.

Conclusion: Apopitotic cumulus cells, TOS levels were found increased for PCOS patients. Also, apopitosis of cumulus cells, were found to be correlated with TOS levels. This correlation was interpreted with improper environment for oocyte maturation.

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

INTRACYTOPLASMIC SPERM INJECTION CYCLE (ICSI) OUTCOME IN PATIENTS UNDER 35 YEARS OLD WITH DIMINISHED OVARIAN RESERVE PLUS SEVERE MALE FACTOR

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Objective: We aimed to evaluate intracytoplasmic sperm injection (ICSI) outcome in young patients (<35 years) with DOR plus severe male factor (SMF) comparing with age- matched controls with DOR.

Material method: This was a retrospective clinical trial, including a total of 189 patients under 35 years-old undergoing ICSI procedures, 35 were infertile couples with DOR plus SMF (the DOR+SMF group) and 154 infertile controls with only DOR (the DOR group).

ICSI success was defined as fertilization rate (FR), development of embryos up to blastocyst stage (day 4 or 5 embryo number/retrieved cumulus oocyte complex (COC) and day 4 or 5 embryo number/ fertilized oocyte number), implantation (positive pregnancy test), clinical pregnancy (CPR) (presence of gestational sac with fetal heart beat), abortion (AR) and live birth rate (LBR) (having a viabl fetus).

Gestational outcome was investigated via evaluating gestational complications (placenta previa, gestational diabetes, gestational hypertension, etc) and delivery type (Caesarian or vaginal delivery).

Gestational week at delivery, anthropometric measurements, requirement of neonatal intensive care unit (NICU) and the presence of congenital anomaly were evaluated as perinatal and neonatal outcomes.

Results: Demographic features were distributed homogenously between the groups. Only the duration of infertility was significantly longer in DOR+SMF group compared to DOR group (p=0.02). Cycle characteristics, oocyte retrieval parameters and fertilization rate were similar between the groups. Embryo number, day 4 or 5 embryo number/COC, day 4 or 5 embryo number/fertilized oocyte number, embryo transfer day and transferred embryo number were similar among the groups (Table 1). When only patients having <1 million sperm/ml were compared to DOR cases, the number of top quality embryos in day 5 was also significantly lower in DOR+SMF group compared to DOR group (p=0.03).

Embryo transfer was performed in 173 of the 189 women in whom ICSI was performed. Implantation was occurred in 84 cases, clinical pregnancy was observed in 67 cases and live birth was observed in 61 cases. Implantation, CPR, LBR and AR weren't significantly different between the groups. Gestational complication, delivery type and congenital anomaly per delivery were distributed homogenously between the groups (Table 2).

A total of 87 gestational sac with fetal heart beat was observed among 84 pregnancy test positive cases and 79 neonate was observed from 87 clinical pregnant cases. Clinical abortion and LBR per gestational sac was similar between the groups. The height and weight of neonate was significantly lower in DOR+SMF group than in DOR group (p=0.001 and p=0.012, respectively). Gestational week at delivery was also lower in DOR+SMF group compared to DOR group (p<0.0001). The requirement of NICU was similar between groups, however, the hospitalization day in NICU in DOR+SMF group was longer compared to DOR group (p=0.023). Congenital anomaly rate per neonate was also similar between groups (Table 3).

Conclusion: Although, the presence of a severe sperm factor in addition to DOR does not affect ICSI outcome, it seems to have a negative effect on perinatal and neonatal outcomes. Only having <1 million sperm/ml may be related to lower top quality embryo number.

Keywords: Diminished ovarian reserve, severe male factor, ICSI outcome

Table 1. Comparison of demographic features and cycle characteristics of groups

	DOR (n=154)	DOR (+) SMF (n=35)	p value
Maternal age (yrs)	32.09 ± 3.03	31.77 ± 3.33	0.5
Paternal age (yrs)	35.31 ± 4.7	35.51 ± 4.69	0.8
BMI (kg/m^2)	25.64 ± 3.98	26.49 ± 3.72	0.2
AMH	0.61 ± 0.61	0.87 ± 0.88	0.1
Duration of infertility (yrs)	5.93 ± 4.02	7.68 ± 4.21	0.02
Cycle (if performed, n)	0.82 ± 1.2	1.25 ± 1.4	0.06
Induction protocol (n, %) Antagonist Long Naturel Seminaturel	148/154 (96.1%) 5/154 (3.2%) 0 1/154 (0.6%)	34/35 (97.1%) 0 1/35 (2.9%) 0	0.1
Total hMG dose	2196.15 ± 1474.37 (n=65)	2059.09 ± 1739.08 (n=11)	0.7
Total FSH dose	1951.47 ± 697.38 (n=127)	1980.46 ± 806.51 (n=32)	0.8
Trigger (n, %) hCG Dual	122/154 (79.2%) 32/154 (20.8%)	23/35 (65.7%) 12/35 (34.3%)	0.1
EMT at trigger day (mm)	9.82 ± 1.77	9.19 ± 1.52	0.053
E2 level at trigger day	805 ± 437.9	804.17 ± 444.1	0.9
Oocyte retrieval parameters COC (n) MII (n) MI (n) GV (n) Oocyte with anomaly (n)	3.46 ± 1.28 2.82 ± 1.16 0.18 ± 0.38 0.42 ± 0.7 0.05 ± 0.32	3.31 ± 1.32 2.68 ± 1.2 0.22 ± 0.68 0.4 ± 0.6 0	0.054 0.5 0.5 0.8 0.2
Fertilized oocyte (n)	2.42 ± 1.14	2.2 ± 1.18	0.2
FR (%)	86.17 ± 27	82.67 ± 24	0.4
Embryo number (n) D2 total embriyo (n) D3 total embriyo (n) D4 total embriyo (n) D5 total embriyo (n)	2.57 ± 1.09 (n=143) 2.51 ± 1.07 (n=137) 2.69 ± 1.06 (n=72) 2.48 ± 1.06 (n=33)	2.17 ± 1.05 (n=34) 2.21 ± 1.08 (n=33) 3 ± 1 (n=11) 2 ± 0.53 (n=8)	0.058 0.1 0.3 0.2
D4 or D5 embryo/COC	0.67 ± 0.26	0.57 ± 0.19	0.2
D4 or 5 embryo/Fertilized oocyte	0.88 ± 0.25	0.73 ± 0.22	0.058
ET day 2 or 3 day 4 or 5 or 6 day	70/139 (50.4%) 69/139 (49.6%)	23/34 (67.6%) 11/34 (32.4%)	0.07
ET cancellation (n, %)	15/154 (9.7%)	1/35 (2.9%)	0.3
TEN (n, %) SET DET TET	32/139 (23%) 102/139 (73.4%) 5/139 (3.6%)	13/34 (38.2%) 20/34 (58.8%) 1/34 (2.9%)	0.1

DOR: Diminished ovarian reserve; **SMF:** Severe male factor; **BMI:** Body mass index; **AMH:** Antimullerian hormone; **hMG:** Human menopausal gonadotropin; **FSH:** Follicle stimulating hormone: **hCG:** Human chorionic gonadotrophin; **EMT:** Endometrial thickness; **E2:** Estradiol; **COC:** Cumulus oophorus complex; **MII:** Methaphase II; **MI:** Methaphase I; **GV:** Germinal vesicle; **FR:** Fertilization rate; **ET:** Embryo transfer; **TEN:** Transferred embryo number; **SET:** Single embryo transfer; **DET:** Diembryo transfer; **TET:** Triembryo transfer.

Table 2. Comparison of intra cytoplasmic sperm injection outcome and gestational and neonatal outcome per delivery of the groups

	DOR (n=154)	DOR(+) SMF (n=35)	p value
Implantation rate per embryo transfer(n, %)	72/139 (51.8%)	12/34 (35.3%)	0.09
CPR per embryo transfer (n, %)	57/139 (41%)	10/34 (29.4%)	0.2
Abortion (n, %)	20/72 (27.8%)	3/12 (25%)	1
LBR (n, %)	52/72 (72.2%)	9/12 (75%)	1
Multiple pregnancy (n, %)	14/72 (19.4%)	4/12 (33.3%)	0.2
Gestational complication (n, %)	12/52 (23.1%)	4/9 (44.4%)	0.2
Delivery type (n, %) Caesarian section Vaginal delivery	48/52 (92.3%) 4/61 (7.7%)	9/9 (100%) 0	1
Congenital anomaly per delivery (n, %)	3/52 (5.8%)	0	1

DOR: Diminished ovarian reserve; **SMF:** Severe male factor; **CPR:** Clinical pregnancy rate; **LBR:** Live birth rate.

Table 3. Comparison of perinatal and neonatal outcome per neonate of the groups

	DOR (n= 66)	DOR (+) SMF (n=13)	p value
Height (cm)	48.56 ± 3.25	44.92 ± 4.21	0.001
Weight (gr)	2949.09 ± 673.19	2380 ± 970.31	0.012
Gestational week at delivery	36.74 ± 2.23	33.69 ± 3.66	<0.0001
Clinical abortion per gestational sac (n, %)	7/73 (9.6%)	1/14 (7.1%)	1
LBR per gestational sac (n, %)	66/73 (90.4%)	13/14 (92.9%)	1
Sex (n, %) Female Male	24/66 (36.4%) 42/66 (63.6%)	5/13 (38.5%) 8/13 (61.5%)	1
NICU (n, %)	11/66 (16.7%)	3/13 (23.1%)	0.6
NICU day	13.27 ± 11.03 (n=11)	41.33 ± 32.33 (n=3)	0.023
Congenital anomaly per neonate (n, %)	3/66 (4.5%)	0	1

THE ROLE OF SYSTEMIC AND LOCAL OXIDANT-ANTIOXIDANT STATUS ON EMBRYO QUALITY AND INTRACYTOPLASMIC SPERM INJECTION-EMBRYO TRANSFER SUCCESS IN UNEXPLAINED INFERTILE WOMEN

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Objective: Oxidative stress has been blamed for the poor intracytoplasmic sperm injection- embryo transfer (ICSI-ET) outcome in infertile patients and ICSI-ET success rate of UI patients is lower than patients with known infertility. Total oxidant status (TOS), total antioxidant status (TAS) and oxidative stress index (OSI) (TOS/TAS) provide a broad overview of redox status. We aimed to investigate the significance of serum (s) and follicular fluid (FF) TAS, TOS, and OSI levels in the prediction of embryo quality and ICSI-ET success in UI patients.

Material Method: 20 UI patients and 20 controls (infertile couples with male factor except azoospermia and severe oligoasthenosperima) undergoing fresh ICSI-ET cycle were recruited. Exclusion criteria were endocrinopathy, chronic disease or medication use, couples who had received any form of vitamin supplementation within 3 months before treatment. Ovarian stimulation was made with short antagonist protocol. FF samples aspirated from mature follicles and blood samples were collected at oocyte retrieval day and TAS, TOS levels were measured and OSI was calculated. The embryo(s) were classified based on their morphological appearance (Table 1). The success of ICSI- ET was defined as implantation (a positive β-hCG 14 days), clinical pregnancy (presence of a gestational sac with fetal heart pulsation) and living birth rate (birth of a viabl fetus).

Results: The median age was higher in the UI group than the control group (p=0.01). There was no significant difference in terms of other demographic features, cycle characteristics, embryo quality and the success rate of ICSI-ET (implantation, clinical pregnancy and live birth rates) between UI and control groups. The demographic and cycle features and serum and follicular TAS, TOS and OSI were similar except estradiol levels between good and poor quality embryos. Estradiol levels were higher in patients with good quality embryos than patients with poor quality embryos (Table 2).

FF-TAS showed a significant negative correlation with age; FF-TOS, FF-OSI, and s-TAS showed a positive correlation with BMI; and FF-OSI showed negative correlation with gonadotrophin dosage per day among the UI group and the whole group. Total embryo scores weren't correlated with oxidative parameters among UI group and the whole group. However, embryo fragmentation and the stage of development scores were negatively correlated with s-OSI and s-TOS. Oocyte retrieval parameters and the number of the fertilized oocytes weren't correlated with follicular and serum TAS, TOS, and OSI levels.

FF-OSI was negatively associated with embryo quality in the UI group (but not in the whole group) after age-adjustment (p=0.04). The negative effect of FF-OSI in relation to embryo score was also observed in the UI group (not in the whole group) (p=0.03) (Table 3). No significant effect of TAS, TOS, and OSI was observed on implantation, clinical pregnancy and live birth rate in UI and the whole group.

Conclusion: Our study reveals an association of local oxidant parameters with the embryo quality in UI patients. High FF-OSI can decrease the quality of embryo in patients with UI.

Keywords: TAS, TOS, OSI, embryo quality, unexplained infertility

Table 1. Comparison of demographic features, cycle characteristics, and treatment outcomes of good and poor quality embryos

	Poor quality embryos (n=17)	Good quality embriyos (n=23)	p değeri
Age (years)	29.11 ± 6.25	27.95 ± 5.19	0.5
Partner's age (years)	33.94 ± 5.973	30.95 ± 5.06	0.09
BMI (kg/m2)	27.09 ± 4.36	25.85 ± 5.25	0.4
Duration of infertility (years)	6.05 ± 4.95	4.76 ± 2.69	0.2
Cycle count (if performed) (n)	1.35 ± 0.6	1.26 ± 0.54	0.6
Day 3 hormone levels FSH (mIU/mL) LH (mIU/mL) E2 (pg/ml) PG (ng/ml)	6.93 ± 2.36 4.48 ± 2.26 33.52 ± 4.38 0.84 ± 0.45	6.92 ± 1.91 5.49 ± 2.45 48.27 ± 15.09 0.92 ± 0.99	0.9 0.1 0.003 0.7
Smoking (n, %)	2/17 (%11.8)	1/23 (4.3%)	0.5
Etiology of infertility (n, %) Unexplained Male factor	9/17 (%52.9) 8/17 (%47.1)	11/23 (%47.8) 12/23 (%52.2)	1
Type of gonadotrophins (n, %) r-FSH u-FSH+r-FSH	11/17 (%64.7) 6/17 (%35.3)	8/23 (%34.8) 15/23 (%65.2)	0.1
Duration of COS	9.7 ± 1.4	9.65 ± 1.46	0.9
Total gonadotropin dose (IU)	2894.05 ± 1100.67	3470.65 ± 1347	0.1
Gonadotropin dose per day (IU)	293.04 ± 87.85	350.19 ± 98.82	0.06
Oosit retrieval parameters Total oocyte number (n) MII rate (%)	9.41 ± 4.73 79.68 ± 17.17	12 ± 5 75.68 ± 14.66	0.1 0.4
Fertilized oocyte (n)	3 ± 1.73	5.26±3.29	0.01
Follicular levels FF-TAS (mmol Trolox Eq/L) FF-TOS (µmol H2O2 Eq/L) FF-OSI (arbitrary unit)	0.99 ± 0.25 9.21 ± 6.26 0.9 ± 0.512	1.05 ± 0.14 7.69 ± 5.06 0.7±0.38	0.3 0.3 0.1
Serum levels s-TAS (mmol Trolox Eq/L) s-TOS (µmol H2O2 Eq/L) s-OSI (arbitrary unit)	1.17 ± 0.12 8.03 ± 6.96 0.67 ± 0.57	1.14 ± 0.11 7.23 ± 4.54 0.62 ± 0.37	0.4 0.6 0.7

BMI: Body mass index; **FSH:** Follicle stimulating hormone; **LH:** Luteinizing hormone; **E2:** Estradiol; **PG:** Progesterone; r: recombinant; u: urinary; **COS:** Controlled ovarin stimulation; **MII:** Metaphase II; **FF:** Follicular fluid; **s:** Serum; **TAS:** Total antioxidant status; **TOS:** Total oxidant status; **OSI:** Oxidative stress index.

Table 2. Parameters and scores used for embryo grading

		0	1	2
Day 5 embryo	Intra cellular mass (ICM); Trophectoderm; Stage of development	Difficult to discern, with few cells; Very few cells; Early	Easily discernible, with many cells that are loosely grouped together; Few cells forming a loose epithelium; Blastocyst	Prominent, easily discernible, with many cells that are compacted and tightly adhered together; Many cells forming a cohesive epithelium; Expanded/ hatched/hatching
Day 4 embryo	Compaction; Fragmentation	Cell size not stage specific; >25%	Incomplete; 10–25%	Complete; <10%
Day 3 embryo	Cell size; Fragmentation	Cell size not stage specific; >25%	Uneven; 10-25%	Even; <10%

***For day 3 and 4 embryos:** embryos with higher than 2 points (>2) were defined as good quality embryos; the embryos with 2 or less than 2 points (<=2) were dfined as poor quality embryos.
***For day 5 embryos:** embryos with higher than 3 points (>3) were defined as good quality embryos; embryos with 3 or less than 3 points (<=3) were defined as poor quality embryos.

Table 3. The association between embryo score and oxidative markers in unexplained group

	Standardized Coefficients (β)	t	p value
Constant		3.21	0.005
s-TAS	-0.41	-1.9	0.07
FF-TOS	1.1	1.97	0.06
FF-OSI	-1.24	-2.28	0.03

Covariates: Age.
FF: Follicular fluid; **s:** Serum; **TAS:** Total antioxidant status; **TOS:** Total oxidant status; **OSI:** Oxidative stress index.

THE EFFECT OF RECURRING STEM CELL APPLICATION ON IMPLANTATION IN EXPERIMENTAL INTRAUTERIN RAT MODEL

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[3]Manisa Celal Bayar University Faculty of Medicine - Department of Histology- Embryology/Manisa/ Turkey

Aim: Asherman syndrome (AS) is a state of both pregnancy complications and intrauterine adhesion (IUA) that may occur after excessive gynecological operations. Because of its increasing prevalence in recent years, the search for alternative treatment continues. Implantation is a set of events that initiate pregnancy by entering the mother's endometrium of the zygote in the blastocyst stage. Many molecular and cellular mechanisms, both from the endometrium and blastocyst, have been reported to play an active role in this complex process. In this study, the AS model, which identifies the formation of intrauterine adhesion in rats, was chemically generated and treated with bone marrow-derived mesenchymal stem cells isolated from the tibia and femoral bone of male rats of the same species and the changes occurring in the implantation stage were evaluated with histopathological parameters. Ethical approval was obtained from the Experimental Animals Ethics Committee of Manisa Celal Bayar University.

Materials-Methods: Intrauterine adhesion model is chemically formed in the single horn of the uterus and treated; Four different groups were tried to be provided with medium (BY), bone marrow-derived mesenchymal stem cells (BMDSC) and 48-hour medium (Niche). The first group (G1); Asherman + BY, second group (G2); Asherman + Niche, third group (G3); Asherman + BMDSC and the fourth group (G4); Asherman + BMDSC + Niche. Animals were sacrificed on the 12th day in order to determine the implantation.

Results: There was a statistically significant difference ($p<0,05$) in the morphological evaluation of the groups according to the number of both histological and implantations in the groups with BMDSC and BMDSC + niche. It was found that niche with BMDSC could remove adhesions in uterine adhesions and make the endometrium more suitable for implantation.

Discussion: Our study showed that many parameters were similar and the implantation rate was higher than other studies when compared experimental formation of uterine adhesion and treatment with various agents.

Support: No financial support was received for this study. Keywords: Stem cell, Infertility, Intrauterine adhesion, Embryology

Besiyeri uygulanan grubun uterus yapısı

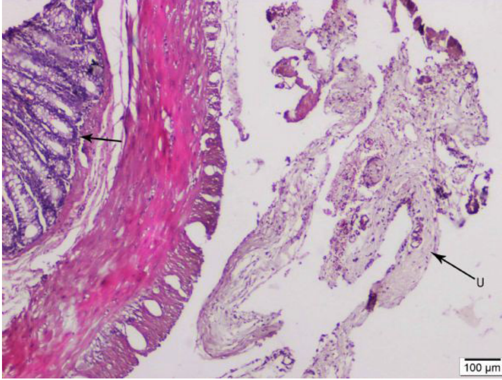

Figure 1. Kalın bağırsakla adezyon meydana gelmiş.

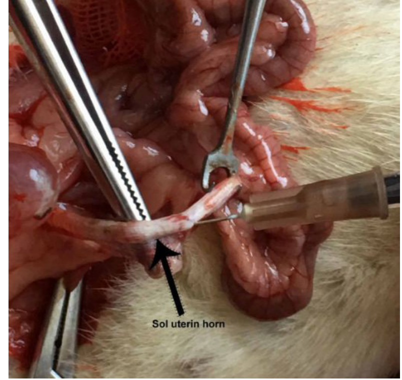

Figure 2. İntrauterin adezyon modeli

Kemik iliği kaynaklı mezenkimal kök hücrelerin stro-1 boyaması

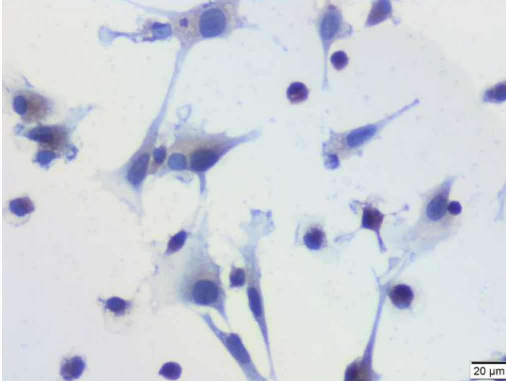

Figure 3. KİMKH'lerin Stro-1 antikor ile boyanmış pozitif immunohistokimya görüntüleri.

Table 1. Grupların sağ ve sol hornundaki implantasyon sayıları

	BY G1-1	NİŞ G2-2	KİMKH G3-2	KİMKH+NİŞ G4-2
Sol horn implantasyonu	0,33 ± 0,5	0	1,5 ± 0,57	3,2 ± 0,83
Sağ+ sol horn implantasyonu	4,33 ± 0,57	4,75 ± 0,5	6 ± 0,81	8,2 ± 0,83

DOES THE TIMING OF ICSI POST CUMULUS REMOVAL WOULD HAVE A CRITICAL ROLE IN CLINICAL PREGNANCY RATES AT ART CYCLES?

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Objective: The aim of this study was to evaluate retrospectively whether cumulus removal performed at different durations in ART patients admitted to Hacettepe University IVF clinic center between January 2016 and January 2018 has an effect on clinical pregnancy rates.

Materials-Methods: This retrospective study was carried out from January 2016 to January 2018. A total of 442 women (aged <= 35 years) undergoing oocyte retrieval procedure for intracytoplasmic sperm injection (ICSI) at Hacettepe University IVF clinic center, were included in the study. All embryos were cultivated within multi-gas incubators (Origio) at 37°C, 5% 02, 5.5% CO2 until the transfer day (3 or 5). Culture was performed without refreshment in 40 µl of single-step medium (One-Step, SAGE) up to blastocyst stage. Two groups were under study. In group A ICSI was performed after cumulus removal in 30 minutes. In group B ICSI was performed after 30 minutes after cumulus removal. Cumulus removal was performed within two hours after oocyte pick up in all groups.

Controlled ovarian hyperstimulation was stimulated with the same protocol (rFSH fixed GnRH antagonist protocol) in each patient. After at least one follicle reached to

>17mm, hCG triggering were performed. Paired samples statistics were used for laboratory outcome comparisons. Chi squared tests were used to compare clinic pregnancy rates between groups. Data presented as mean ± standard deviation. Exclusion criteria included patients with severe male factor and cycles without embryo transfer.

Results: The mean age (32.1±5.2, 32.5±5.2; p=.429), AMH level (2.81±3.2, 3.19±3.2; p=.254) values were not different statistically between two groups. In the selected patient groups, mean values of time between the initiation of ICSI after cumulus removal were found to be respectively; 10.5±7.3, 110.70±51.5; p<0.001. MII oocytes (5,14±3.0, 5,93±3.5; p=.014) and 2-PN number of oocytes (3.79 ± 2.29, 4.36 ± 2.70; p=.019) were significantly higherin group >30 min groups. However, no significant difference was observed in terms of clinical pregnancy (35.0%, 43.0%; p=.091).

Discussion: Each oocyte is surrounded by a cell cluster called the cumulus-corona-oocyte complex. Granulosa cells in the cluster are embedded in long hyaluronan oligosaccharide chains cross-linked by a complex of extracellular matrix proteins and proteoglycans. This cluster of cells must be removed prior to ICSI to visualize, grade and inseminate the oocyte. The cumulus cells play a critical role in oocyte maturation, ovulation and fertilization. However, prolonged culture duration with cumulus cells induces the apoptotic changes in oocytes. According to current study, it was predicted that clinical pregnancy rates were not difference in IVF patients who underwent ICSI within or with 30 minutes after cumulus excretion. Therefore it seems that both options are acceptable.

Keywords: ICSI, ART, cumulus, oocyte, pregnancy

Table 1. Mean age, AMH, MII oocyte, 2-PN, clinic pregnancy values of the patients. All data were expressed as mean ± SD (standard deviation)

	Group A(n=263)	Group B (n=179)	p* Value
Age	32.2 ± 5.2	32.6 ± 5.2	0.429
AMH	2.81 ± 3.2	3.19 ± 3.2	0.254
MII oocyte	5.14 ± 3.0	5.93 ± 3.5	0.014
2-PN	3.79 ± 2.3	4.36 ± 2.7	0.019
Clinic Pregnancy	35.0%	43.0%	0.091

PLACENTAL TOXIC METAL AND TRACE ELEMENT LEVELS OF FERTILE WOMEN

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Objective: The aim of this study is investigation of induvidual effects to cadmium, lead, zinc, and copper levels in human placenta of fertile women.

Material and Methods: Placental samples were obtained from 80 fertile women at the time of delivery. Metal levels were determined by flame atomic absorption spectrometry.

Results: Average lead, cadmium, zinc and copper levels in placental tissue were found as 10.82±1.17 ng/g, 33.07±1.79 ng/g, 23.14±1.35 ~g/g and 3.89±0.16 ~g/g, respectiveley. Smokers had significant1y higher cadmium and lead concentrations in placenta ($p<0.05$) than non-smokers. Placental cadmium and lead levels were significanty increased with mothers age. Furthermore, cadmium levels were found as statistically significant with increasing gestation age ($p<0.05$).

Conclusion: In this study it was presented eventhough Ankara is not an industrial region, there is environmental exposure to toxic metals when considering the metal accumulations in placenta of fertile women. As a consequence it was concluded that polymorphism and expression of metal transporting proteins and the levels of other toxic metals should be investigated. These results should also be compared with the results of IVF patient's placental Toxic Metal and Trace Element Levels level in further studies.

Key words: Cadmium, Lead, Zinc, Copper, placental tissue, AtomicAbsorption Spectroscopy.

INVESTIGATION OF THE EFFECT OF APOPTOTIC INDEX ON OOCYTE QUALITY IN CUMULUS CELLS OF POLYCYSTICOVER PATIENTS IN IVF TREATMENTS

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Selection of quality embryos for transfer in assisted reproductive techniques is critical. The formation of a quality embryo, implantation and the success of pregnancy depend on the quality of oocytes. The selection of the appropriate oocyte for insemination is based on subjective morphological criteria such as growth rate, early cleavage, degree of fragmentation, blastocyst formation. However, morphological assessment alone does not precisely determine oocyte adequacy. Therefore, it is thought that the development of objective, accurate, rapid, cost-effective and non-invasive methods to determine oocyte quality may increase the chance of successful pregnancy and decrease the number of embryos to be transferred.

Cumulus and granulosa cells surrounding the oocyte play a dominant role in the regulation of cell functions during follicle development and are thought to indirectly reflect oocyte quality due to an increase in apoptosis in the cumulus cells after ovulation. Therefore, determination of apoptosis in cumulus cells may provide a non- invasive method for identifying quality oocytes, fertilizing and determining pregnancy success.

As a result of this study, it can be concluded whether the apoptotic index of cumulus cells in PCOS patients will have an effect on determining oocyte quality, and will contribute to quality oocyte selection as a non-invasive method in IVF applications of patients with infertile PCOS and other studies to be done in this regar.

This study was planned as a descriptive study to investigate apoptosis rates in cumulus cells of women with PCOS by immunohistochemical methods, to compare reactive oxygen derivatives (ROS) levels in follicular fluids and to correlate with oocyte quality.

Study group: Female patients admitted to IVF treatment with PCOS (n = 15)

Control group: Female patients with unexplained infertility-induced infertility who were treated with IVF (n = 15).

Keywords: oocyte quality, apoptosis, oxidative stress

COMPARISON OF PI3K-AKT PATHWAY PROTEINS EXPRESSION LEVELS AND IMMUNOLOCALIZATIONS IN NORMOZOOSPERMIC AND OLIGO/ASTHENO/TERATOZOOSPERMIC SPERM SAMPLES

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Aim: PI3K-Akt pathway is an intracellular signal transduction pathway that promotes metabolism, proliferation, cell survival, growth and angiogenesis in response to extracellular signals. This is mediated through serine and/or threonine phosphorylation of a range of downstream substrates. Key proteins involved are phosphatidylinositol 3-kinase (PI3K) and Akt/Protein Kinase B. Phosphoinositide 3-kinase (PI3K) activity has been reported to be critical to sperm motility and mitochondrial ROS generation. The key molecules involved in this signaling pathway are receptor tyrosine kinase (RTKs), phosphatidylinositol 3-kinase (PI3K), phosphatidylinositol-4,5-bisphosphate (PIP2), phosphatidylinositol-3,4,5-bisphosphate (PIP3) and AKT/protein kinase B.

We aimed to compare the expression levels and immunolocalization of PI3K, Akt and p-Akt proteins in normozoospermic samples with non-normozoospermic (oligo/astheno/teratozoospermic) samples to analyse the effect of this pathway on sperm parameters.

Materials-Methods: Semen samples of 24 couples were collected who underwent IVF treatment because of infertility. Samples were collected by masturbation after 3-7 days of sexual abstinence and semen analysis were performed according to the World Health Organization semen analysis guide (WHO, 2010).

Immunohistochemistry was performed to analyse the expression levels and immunolocalization of PI3K, Akt and p-Akt proteins. Localization of proteins in sperm cells were determined and classified as total head, equatorial, posterior anterior, midpiece, tail membrane and cytoplasmic droplet. At least, 100 sperm cells were counted from each patient and the results were given as %.

Results: P13K and Akt expression rates were not different between normozoospermic and non-normozoospermic samples (p= 0,931; p=0,471 resp.), although p-Akt expressions which is the activated form of Akt were significantly increased in non-normozoospermic samples (p=0,047). All three pathway proteins were expressed in the entire sperm cell but immunolocalization is detected especially in midpiece and tail regions.

Conclusion: P13K pathway which controls many cellular responses in sperm cells were expressed in all parts of the sperm cell. p-Akt expressions which is the activated form of Akt were significantly increased in non-normozoospermic samples indicating the possible role of this protein in spermatogenesis and thus sperm parameters.

Keywords: PI3K pathway, sperm, normozoospermia, Akt, p-Akt

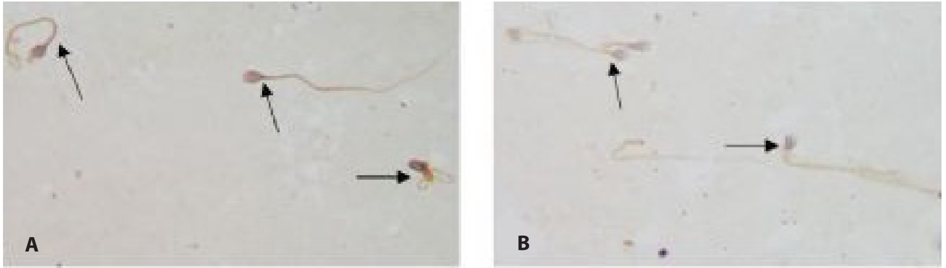

Figure 1. p- Akt expressions in normozoospermic (A) and non-normozoospermic (B)samples

OXIDATIVE STRESS DETERMINED BY OXIDATION–REDUCTION POTENTIAL, IS AN INDICATOR OF REDUCED SPERM PARAMETERS

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Objective: Oxidative stress (OS) is one of the key reasons that has been shown to have an important role in male infertility. OS occurs when there is an imbalance between reactive oxygen species (ROS) production and the antioxidant mechanisms that scavenge them. Increased levels of ROS causes peroxidation of lipids, damage to proteins, DNA damage, apoptosis, which causes decreased sperm function and reduced fertility A novel measurement method, the MiOXSYS system, that comprises all oxidants and antioxidants together is developed to measure all oxidation–reduction potential (ORP) of semen samples. An ORP cut-off value of 1.36 mV/106 sperm/ml is accepted to predict abnormal semen quality according to the manufacturer. The goal of this study was to investigate the correlation of sperm parameters with the oxidative stress according to the reference value of ORP levels measured with MIOXYS.

Materials and Methods: Semen samples of 121 patients that applied for infertility evaluation at Medistate Hospital, IVF Center between August 2018 and April 2019 were included in the study. Semen analysis were performed according to the WHO 5th edition guidelines (World Health Organization, 2010). The patients were divided into two groups according to the cut-off value (1.36 mV/106 sperm/ml) of ORP levels: First group patients OS levels were above the cut-off value (OS +) (n: 39) and second group below the value (OS –) (n: 82). Semen parameters (volume, sperm concentration, total motility, progressive motility, immotile sperm) were compared between the groups. ORP levels was measured in millivolts (mV) using MiOXSYS System (Aytu Bioscience, Englewood, USA). Raw sORP values (mV) were normalized to sperm concentrations and sORP values were presented as mV/106 sperm/mL. Data are presented as mean ±SD. Comparisons of groups were performed using chi-squared test for categorical variables. A Wilcoxon rank-sum test was used for the comparisons of quantitative variables. Tests were performed at a significance level of $p < 0.05$.

Results: Sperm parameters according to ORP levels were given in Table 1. Sperm concentration (mil/mL), total motility rate (%) and progressive motility rate (%) were significantly lower in ORP + patients (n = 39) compared to ORP - (n = 82) ($p < 0.000$) while immotile sperm rate (%) were significantly higher in ORP + patients (Table 1, Figure 1). Semen volume were not effected from the ORP levels.

Results of the recent study confirmed that ORP levels measured with MIOXYS is significantly correlated with sperm parameters and may be used as an indicator of sperm parameters. It may also concluded that the reference value determined by Agarwal et al is a reliable value that may distunguish the normal semen parameters with reduced ones.

The authors declare no conflict of interest and no financial support

Keywords: Male infertility, MIOXYS, Oxidative stress

Table 1. Semen parameters according to oxidation-reduction potential (ORP) Values are presented as mean

Variable	OS (+) (n=39)	OS (-) (n=82)	p-value
Static ORP level (mV)	65,22 (13,8-337,5)	23,27 (0,1-70,7)	0,000
ORP (mV/106 sperm/mL)	3 (2–3.75)	3 (2–4)	0,000
Volume (mL)	16,71 (1,8–84)	49,15 (1,1-210)	0,342
Concentration (x106 sperm/mL)	16,71 (1,8–84)	49,15 (1,1-210)	0,000
Total motility (%)	49,74 (0-82)	59,9 (8-88)	0,003
Progressive motility (%)	4,53 (0–23)	11,92 (0-39)	0,000
Immotile sperm (%)	50,0 (12–100)	40,19 (12-92)	0,004

Figure 1. Sperm parameters in OS (+) and OS (-) semen samples Discussion

HOW TO COUNSEL WOMEN WHEN THERE IS ONLY ONE SLOW-GROWING DAY 5 EMBRYO WITH NO OTHER EMBRYOS AVAILABLE FOR CRYOPRESERVATION?

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Study Question: Is pregnancy rate different when a single blastocyst at different stages of development is transferred with no excess embryos available for cryopreservation.

Summary Answer: In women who have no excess embryos for cryopreservation, transfer of a single blastocyst at the early or expanded stages yields similar, while transfer of a cavitating morula is associated with very low pregnancy rates.

What is known already: Transfer of slow growing embryos, when the culture period is extended, and frozen thereafter, yield satisfactory pregnancy rates. However, the best approach is not known when there is only one slow-growing embryo on day 5.

Study Design Size, Duration: This retrospective cohort study, performed between January 2016 and January 2018 in single private IVF Centre setting included 86 mandatory single fresh Day 5 embryo transfer cycles with no excess embryos available for cryopreservation.

Participiants/materials, setting, Methods: Patients with a single Day 5 embryo scheduled for fresh embryo transfer were grouped according to rate of expansion of blastocoel cavity; Group I,morula and cavitating morula(n:24), group II early blastocyst (n: 32), Group III expanded and hatching blastocyst (n:30). None of the patients had excess embryos suitable for cryopreservation.

Main Results and Role Of Chance: Female age, duration of infertility, total gonadotropin consumption were similar between the groups (overall 33.5± 5.5 years, 24 months and 3215± 1395 IU). Number of oocytes collected, metaphase II, 2PN and cleavage stage embryos were significantly less in Group I compared to Group II 6.5 ±3.4, 4.2±2.5, 3.17±2.3, 3.13±2 versus 11.6±5.5, 8.03±3.9, 5.88±3.3, 6,13±3.5) but was similar with Group III. (8.3±4.5, 6.47±4, 5.1±3.5, 5.1±3.7). Clinical pregnancy rates and live birth rates in group I was significantly lower than Group II and III which were 12.5% vs 31.3% and 50% (p=0.015) and 4.2% vs 18.8% and 33.3% respectively (p=0.02).

Limitations and caution: Retrospective design of the study is its major limitation. No information could be drawn from the study whether extending culture in morula and cavitating morula embryos and freeze/thaw of the expanded blastocyst would yield better LBR

Wider implications of the Findings: Fresh transfer of early blastocyst results in similar outcomes to the transfer of fully expanded blasyocysts. When only morula/cavitating morula embryo are present, the couple should be counselled regarding the very low chances of a live birth rate.

Keywords: blastocyst, embryo, morula

Poster Presentations

CORRELATION OF OUTCOME OF TESTICULAR SPERM EXTRACTION PROCEDURES WITH SIMULTANEOUS PATHOLOGY REPORTS

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Study Question: Are pathology reports of the testicular sperm extraction (TESE) procedures in concordance with the outcome encountered in the embryology laboratory

Summary Answer: There is discordance in a striking number of cases between the embryology results and the pathology reports.

What is Already Known: The introduction of intracytoplasmic sperm injection (ICSI) has revolutionized the treatment of male-factor infertility. Even with a single spermatozoon a pregnancy can be achieved. However, histopathology reports do not always correlate with those of TESE results of the embryology lab. In addition to the importance of bilateral biopsies to gain optimal diagnostic and therapeutic results, meticulous evaluation of testicular tissue samples have utmost importance since overlooking a single spermatozoon by the embryologist may result in the false decision of not to enroll the couple in ICSI program while a false negative report of the pathologist does not affect the treatment decision.

Study Design, Size, Duration: Medical records of 250 infertile couples who presented to our center between 1 February 2009 and 31 July 2017 and diagnosed with azoospermia were reviewed retrospectively.

Participants/Materials, Setting, Methods: TESE procedures were done under microdissection microscope. The results of the embryology lab were reported as either positive (existence of spermatozoa qualified for ICSI) or negative (existence of either immature forms of sperm cells or no sperm cells at all). The pathology results were reported in accordance with the Johnsen scoring system.

We compared the embryology lab results with those of simultaneous pathology reports.

Main Results and The Role of Chance: Karyotype analyses revealed abnormal results in 26% of the patients. Y chromosome microdeletion was found in 4% of the cases. All TESE negative results of the embryology lab were also reported as negative by the pathologist. However, the agreement of the embryologist and the pathologist in cases reported as positive by the embryology lab was quite poor. 59/122 (48,4%) of the TESE + cases were reported by the pathologist as TESE negative. In only 63/122 (51,6%) TESE positive cases, there was concordance between the embryology result and the pathology report.

Limitations, Reasons for Caution: Retrospective design and small sample size are the limitations.

Wider Implications of The Findings: A conceivable explanation for this discordance may be focal spermatogenesis in non-obstructive azoospermia cases. Besides, very scarce spermatozoa might be overlooked by the pathologist due to the much smaller tissue sample provided for pathological examination. Meticulous assessment of the tissue samples is the cornerstone to avoid false negative results.

Keywords: Testicular sperm extraction, azoospermia, pathological examination

<table>
<tr><th colspan="4">Table 1.</th></tr>
<tr><th></th><th>Pathology -</th><th>Pathology +</th><th>Total</th></tr>
<tr><td>Embryology -</td><td>128 (100%)</td><td>0(0%)</td><td>128</td></tr>
<tr><td>Embryology +</td><td rowspan="2">59 (48,4%)</td><td rowspan="2">63 (51,6%)</td><td>122</td></tr>
<tr><td>Total</td><td>250</td></tr>
<tr><td colspan="4">* Chi square test was used (p<0,05</td></tr>
</table>

CALCIUM IONOPHORE (A23187) SHOULD BE USEFULL OR NOT IN IVF PATIENTS

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Background and Aim: Investigation of the effect of calcium ionophore (A23187) on the pregnancy in various IVF cycles using oocyte count and sperm count parameters in IVF laboratories. Calcium ionophore application is used in various IVF cycles and especially in patients with fertilization problems. The effects of calcium ionophore on clinical pregnancy have always been to discussion.The aim of our study was to investigate the effect of calcium ionophore on the pregnancy in IVF cycles. Methods: 512 IVF cycles between 2016 and 2018 were retrospectively reviewed in the Bahçeşehir University Göztepe Medical Park IVF Center. Patients; Patients who underwent calcium ionophore over the age of 35 were divided into several groups according to the number of oocytes and sperm count parameters. Oocyte count was grouped as patients with a number below 3 and 3. In the same way, sperm counts were grouped as ^{6}1x10x and> 1x10ıları. Oocytes of the patients included in the study were administered a ready-made calcium ionophore (a23187) for 15 minutes immediately after ICSI. The clinical pregnancy rates of the groups were compared after calcium ionophore application.

Results: In the evaluation of calcium ionophore applications;

Group1: A total of 7 patients with oocyte count and sperm count of iyon 1^{2}10 sperm was found to have a 28% clinical pregnancy rate after administration of calcium ionophore after ICSI.

Group 2: A total of 81 patients with oocyte count and sperm count> 1x10 was found to have a clinical pregnancy rate of 17.2% after administration of calcium ionophore after ICSI.

Group 3: A total of 15 patients with oocyte count> 3 and a sperm count of m 1x10 ısı.

Group 4: A total of 40 patients with oocyte count> 3 and sperm count> 1x10 kalsiyum had a clinical pregnancy rate of 40% after administration of calcium after ICSI.

Conclusions: As a result, Ca ionophore (A23187) significantly increased clinical pregnancy rates in women with advanced maternal age, especially in patients with more than 3 eggs. In our study, it was shown that Ca ionophore (A23187) can be used safely in older patients and in other patients.

Keywords: Calcium Ionophore, advanced maternal age, fertilisation failure

AMH OR AFC: WHICH IS SUPERIOR IN PREDICTING OOCYTE NUMBER?

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Introduction: Based on the guideline for controlled ovarian stimulation released by ESHRE as a draft in February 2019 both Anti Müllerian Hormone (AMH) and antral follicle count (AFC) were found to be reliable predictors of ovarian reserve categories. accordingly, practitioners were advised to choose either one depending on the local circumstnces.

Material-Method: Medical records of all patients who were enrolled in IVF treatment between January 2016 and December 2018 were retrospectively reviewed. A total of 212 patients for whom both predictors were used were included in the study. all stimulations were performed in Accordance with antagonist protocol. The relation of AMH and AFC levels with total and mature oocyte numbers were investigated.

Results: To reveal the association of the dependent variable total oocyte number with the independent variables AMH and AFC "Simple Regression Analysis". Following are the results of the ANOVA: F (calculation value) of the model = 145,767 and p= 0,000, showed the coefficients pertaining the the two independent variables were found to be statistically significant (P< 0,05).

Y(oocyte) = a (model constant) + b1(AFC) + b2(AMH) Y(oocyte) = 2,582 + 0,314(AFC) + 1,142(AMH)

Discussion: Even though both variables are reliable in predicting the number of oocytes collected,AMH was found to be statistically significantly better. Anyway, decision to choose one of them should depend on availability and cost effectiveness of the local circumstances.

Keywords: AMH, AFC, oocyte

Table 1. Age and body mass index characteristics of patients

Number of patients	**212**
Average age	35,64±5,97
BMI	24,18±4,25

Table 2. Average AMH and AFC levels regarding to age

		AMH (ng/dl)	AFC (#)	Total oocyte (#)	Total 2PN (IVF+ICSI)
<35 age	89	2,82±2,29	12,33±8,86	10,15±5,74	6,26±4,60
35-40	77	1,75±1,67	8,49±6,85	7,14±6,13	4,34±3,65
>40 age	46	0,83±0,69	4,78±2,74	4,28±2,97	2,63±1,61

Table 3. AMH and AFC levels in ICSI patients and total MII oocyte count

		AMH	AFC	MII oocyte
<35 age	89	2,82±2,29	12,33±8,86	5,31±4,28
35-40	77	1,75±1,67	8,49±6,85	3,55±3,39
>40 age	46	0,83±0,69	4,78±2,74	1,91±1,86

THE EFFECT OF SPERM PREPARATION METHODS ON SPERM CHROMATIN CONDENSATION AND DNA DAMAGE

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In the evaluation of the male factor, standard semen analysis is performed according to WHO(World Health Organization) criteria. However, semen analysis does not provide information about sperm DNA damage and maturation.

The study was submitted to the Biruni University Ethics Committee. Ethics Commite was confirmed study with number 2018-12-9 at 29.01.2018. The study was applied to the Biruni University Hospital between January 2018 and June 2018 in patients who applied for spermiogram analysis. In this study; Standard semen analysis was implemented in normospermia (n: 20) and oligoastenoteratospermia (n: 20) patients who applied to Biruni University Hospital Urology outpatient clinic. There after, Sperm maturation defect with acidic aniline blue method and sperm DNA fragmentation index were determined by acridine orange staining method. After separation of the sperm by using gradient and swim-up methods, the groups were separated from them and incubated at room temperature (22 ° C) or 37 ° C for 1 hour. After incubation, acidic aniline blue staining and acridine orange staining method was applied. After implementation, It was compared predecessor and posterior value. Sperm maturation defect was compared with the values obtained after the application.

In this study, student's t test was used for comparison of two groups and ANOVA-Dunnett t test was used for comparison of more than two groups.

According to this study, there was no significant difference between swim-up and gradient methods in terms of concentration, motility and morphology. Both methods can be preferred according to the patient's condition by considering the motility and concentration parameters. In both methods, sperm immaturation and DNA fragmentation were also decreased ($p<0,001$). Especially in cases of oligoasthenoteratospermia, the incubation at 37 ° C had detrimental effects especially on DNA fragmentation compared to normospermia ($p<0,001$).

In this study it has been shown that the most functional incubation temperature at the swim-up or gradient technique are room temperature (22 °C). Therefore incubation can be carried out at room temperature to minimize sperm DNA damage

Keywords: DNA damage, DNA fragmentation, gradient, swim-up

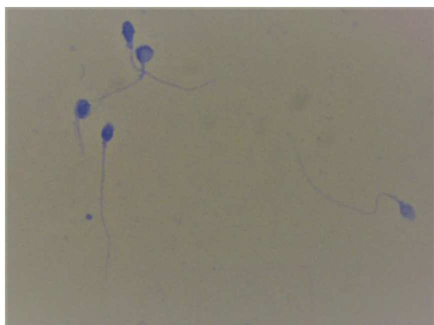

Figure 1. Acidic aniline blue staining

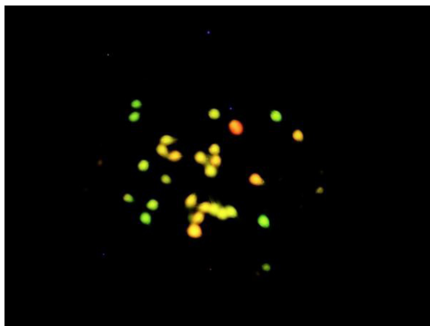

Figure 2. Acridin orange staining

Table 1. Sperm chromatin condensation defect

	Normospermia (x±SD)	OAT (x±SD)	t	p
Before	21,05±7,193	50,55±10,738	-10,207	<0,001
G/RT	16,630±7,027	42,60±9,877*	-9,736	<0,001
G/37	15,35±6,862	40,70±9,587*	-9,616	<0,001
SW/RT	16,75±6,965	46,50±10,216	-10,760	<0,001
SW/37	15,25±7,018	45,50±9,671	-11,3220	<0,001

Table 2. Sperm DNA fragmentation

DFI%	Normospermia	OAT	t	p
Before	21,50±6,605	30,85±10,644	-3,380	0,002
G/RT	18,00±6,224	28,35±10,946	-3,676	0,001
G/37	19,55±6,224	32,55±10,889	-4,518	<0,001
SW/RT	16,60±6,402*	25,50±10,455*	-3,247	0,002
SW/37	19,90±790	29,67±10,23	-3,870	0,001

EXAMINATION OF SPERM CALCIUM CHANNELS AND OLFACTORY RECEPTORS IN VARIOUS INFERTILE CASES IN ODORANT PROGESTERONE APPLIED CULTURE MEDIUM.

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The combination of the egg and the sperm cell is not a process which sperm can only leak into the egg by mechanical processes. In this process, an unusual and special biochemical reaction plays a leading role. Sperm odor receptors are an important factor in the regulation of chemotactic behavior with changes in calcium channels. Ca2 + flow leads to an increase in intracellular calcium concentration and allows sperm to move faster. In addition, Ca2 + channels have been shown to be essential for fertility. Odorant; acting as a chemoattractant, it interacts with the hor17-4 odorant receptor that found in human sperm and causes chemotaxis. Burjonal used as odorant in studies, a strong agonist for odorant receptors and chemoattractants. By using this chemoattractant, we provided important information about the interaction of odor receptors in the sperm.

Some researchers found that progesterone in the rabbit oocyte causes chemotaxis such as bourgeonal, however, human studies were lacking in the ability of sperm odorant receptors and calcium cation channels to reach the egg and perform normal physiology. The aim of this study is investigate the effects of sperm odor receptors on sperm tail movement and to evaluate hyperactivity in fertile and infertile cases in odorant progesterone treated culture media. The study was submitted to the Biruni University Ethics Committee and was approved in 2018/13-6 and dated 26.02.2018. The study was applied to the Biruni University Hospital between January 2019 and June 2019 in patients who applied for spermiogram analysis. In study; patients admitted to the Biruni University Hospital Urology outpatient clinic. Standard semen analysis was performed according to WHO criteria in normospermia (n:28) and oligoastenoteratospermia (n:28) groups, followed by swim up method and by adding progesterone sperm motility examined and with immunofluorescence method changes in sperm calcium channel were evulated. After sperm separation by swim-up method, progesterone was applied and incubation was carried out at 37 ° C for 45 minutes. After incubation, motility was evaluated and pre and post values were compared. The samples were frozen in the hospital and then examined by the immunofluorescence technique for the evaluation of calcium channels by using Catsper antibody and the pre-values were compared. According to this thesis, there was a significant difference in motile sperm between swim-up and progesterone which was used as a sperm preparation method. Motility parameters were taken into account and the changes in calcium channels of progesterone-treated sperms caused hyperactivation. The findings of this study have aimed to compare sperm odorant receptors and calcium channels in infertile and fertile cases and to make a contribution to the current literature in unexplained infertility group.

Key words: Ca2+, Catsper, swim-up, immunofluorescence, odorant

A NEW ISOLATION AND CULTURE METHOD FOR HUMAN GRANULOSA CELLS

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Purpose: Granulosa cells have a critical role in folliculogenesis(1). Histological evaluation of follicular development is performed through microscopic investigation of granulosa cells. The cumulus-oocyte complex is a compact cellular mass, released at ovulation. Cumulus-oocyte complex composed of both oocyte and surrounding cells such as corona radiata and other cumulus cells. Both oocyte and granulosa cells interacts each other by several molecules during folliculogenesis.Thus examining granulosa cells under the microscope have a crucial importance to understand ovarian pathologies.Aim of this study is to provide an optimized method for isolating and culturing granulosa cells from follicular fluid which is obtained during the oocyte pick up(OPU)process and from remaining fluid after oocyte isolation and denudation.

Material–Method: During OPU, obstetrician collected drained fluid which passed to laboratory for the investigation of oocyte-cumulus complexes. This liquid was diluted 50% with dPBS(calcium-magnesium free). Lymphocyte separation media(Lymphosep,Biowest-BW) is added 3cc to each 15cc centrifuge tubes.Later 10ml of diluted follicle fluid is added onto separation medium carefully in order not to mix with medium.After tubes centrifugated at 980G for 45minutes, red pellet appears at bottom and two phases are seen on top. Between middle colorless and uppermost yellowish phases, a nebulous floating cell mass seen clearly. These cell mass and yellowish phase is transferred to another centrifuge tube and diluted with DMEM/F12 basal medium(v/v 1:1).For removing seperation medium from this suspension which centrifuged(at 226G for 5minutes)again and pellet solved with 1ml of DMEM/F12 complet medium (%5FBS,%4L-glutamin,%1penicilin-streptomycin). Vessel will be selected according to number of isolated cells and feeded with 1ml or more BIOAMF-1 medium according to vessel. At 24-48 hours, cells will be confluent.If confluency cannot accomplished, refresh the medium with complete DMEM/F12 medium.

Results: We obtained at least 3 passages of granulosa cells with this method. First we deal bacterial infection which was overcomed by the maximum attention of all staff(obstetrician, embryologists) to sterilization both in vaginal area and working stations.Second we deal with seperation method.Three seperation mediums(Ficoll, Histopaque, Lymphosep)were tried and compared. Lymphosep was the most successful one to isolate granulosa cells. Third we deal with attachment problem. For enhancing the attachment of more cells we used 1:1 complet BIOAMF-1 and DMEM/F12 mixture together. This mixture was not only economic but also successful. All scientists studying with granulosa cells generally have knowledge about the troubles and difficulty in isolation and proliferation of granulosa cells. High FBS and low L- glutamine levels had a reverse effect on proliferation(2). Several growth factors can be used but it is not effective or economic. So we reduced FBS and increased L- glutamine.

Discussion: Sterility is important issue in cell culture. Thus clinicians must pay attention to obtain follicular fluid and oocyte. Moreover an equal amount of follicles and cells cannot be obtained from each patient so we must use appropriate vessels (Table1). Attachment and proliferation is an another limiting procedure to get enough cells. If you can overcome these challenges you will get valuable amount of human cells for experiments from these waste fluids of OPU.

Contribution: Hacettepe University, Scientific Research Unit;Comprehensive Project TSA-2018-17320

Keywords: granulosa cell, isolation, cell culture, ovarian pathologies

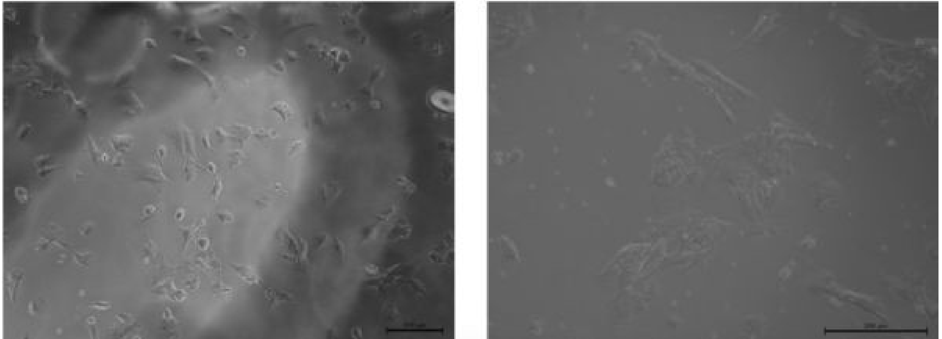

Figure 1. Granulosa cells in culture; left is 10x and right is 20x

Table1. Vessels and seeding densities for granulosa cells	
Vessel	**Seeding density**
6-well plate (one well of plate)	> 500 x 10^3
60 mm petri dish	500 x 10^3 - 1.000 x 10^3
T25 flask	1.000 x 10^3 – 1.500 x 10^3
T75 flask	1.500 x 10^3 – 3.000 x 10^3

IVF RESULTS OF KLINIFELTER SYNDROME AND NORMAL KARYOTYPE NON-OBSTRUCTIVE AZOSPERMIC PATIENTS

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Purpose: To compare IVF results of Klinefelter Syndrome (KS) with normal karyotype infertile men with non-obstructive azoospermia (NOA). Materials-Methods:

This is a retrospective study involving 23 patients who were applied TESE (Testiculer sperm extraction) in the IVF Center Gazi University Faculty of Medicine between 2000-2017. The control group consisted of 51 patients who were non-obstructive azoospermia (NOA) with normal karyotype. FSH, luteinizing hormone (LH), free testosterone and total testosterone levels, karyotype analyzes and TESE pathology results of patients were evaluated.

There were no significant differences between KS and control groups by the means of age of men and women; duration of infertility; number of IVF trials.

Data were evaluated with Statistical Package for Social Science (SPSS version 20, IBM, Chicago). For quantitative analyzes, the qualitative analyzes were compared with the chi-square test and Fisher 's exact tests.

Results: The hormone values of male patients KFS and control groups were as follows; FSH 34 ± 2 and 15.3 ± 11.3 mIU / ml (p: 0.005), LH 23 ± 2.4 and 7.3 ± 6.6 SD mIU / ml (p: 0.006), free testesterone 6.9 ± 3 and 13.5 ± 16.1 pg / ml (p: 0.36), total testosterone 5 ± 3.9 and 5.6 ± 5.2 ng / dl (p: 0.72) and prolactin 8.2 ± 2, 8 and 8.6 ± 4.1 ng / ml (p: 0.77), respectively. In the KFS group, the FSH and LH values is significantly higher than the control group. There was no statistically significant difference between free testosterone, total testosterone and prolactin hormone levels.

In terms of testicular sperm retrieval rate, the rate of finding sperm was found to be 30% and 47% in the control group (p = 018). No statistical difference was found between the two groups in terms of sperm recovery rate with TESE.

Clinical pregnancy rates for both groups were 28.5% for ICSI cycles and 41.6% for control group. The statistical difference between the two groups was significant. While live birth was not achieved in patients with KS in our center, the live birth rate of our control group was 37.5% per ICSI cycle. There was a statistical difference between two groups for live birth (p = 0.002)

Conclusion: There is a no significantly difference with sperm recovery rate between KS and control groups. On the other hand, clinical pregnancy and live birth rate were significantly higher in control group than KS group.

Keywords: Klinefelter Syndrome, TESE, clinical pregnancy

EVALUATION OF SPERM CRYOPRESERVATION AND ITS RESULTS WITH DIFFERENT CRYOPROTECTANT SOLUTIONS

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Cells and tissues are cooled at very low temperature, all biological activities to stop, minimal damage and function without loss of long-term use the purpose of future storage. We investigated the effect of cryoprotectant on DNA fragmentation, maturation, motility in after freezing. Similar studies, it can be improved by making changes in the cryopreservation method with the least amount of sperm DNA damage these changes will minimize the damage of frozen sperm DNA, better quality sperm can be obtained and the success of pregnancy and live birth rate. The study was submitted to the Biruni University Non-Interventional Ethics Committee and was approved by the ethics committee of 2018-12-9 and dated 29.01.2018. The study was carried out between September 2017 - April 2018 in patients applying for spermiogram analysis to Fertijin Women's Health and IVF Center.Among the patients who applied to IVF Center, standard semen analysis was performed according to WHO criteria in normozoospermia (n: 20) and OAT groups (n: 20). Sperm samples were washed using the in gradient, technique as a sperm washing method and were thawed with three different solutions as the cryoprotectant in the wash thecnique. They were thawed after 24 hours before freezing. Then all samples were evaluated with acridine orange and acidic alanine blue painting procedure on about sperm maturation and DNA fragmentation. According to this study, there is no significant difference between in sperm motility, DNA fragmentation and sperm maturation in these sperm samples after thawing or frozen with different cryopreservation solutions.

Keywords: Acridin orange, annilin blue, DNA fragmantation, sperm freezing, sperm maturation

Fluorescence microscopy of sperm stained with acridine orange dye

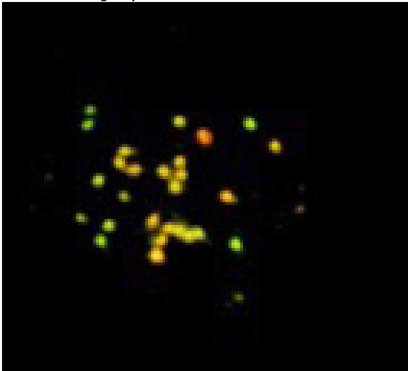

Figure 1. Acridine orange staining shows green (normal) and orange (defective) DNA fragmentation

Light microscopy of sperm stained with aniline blue

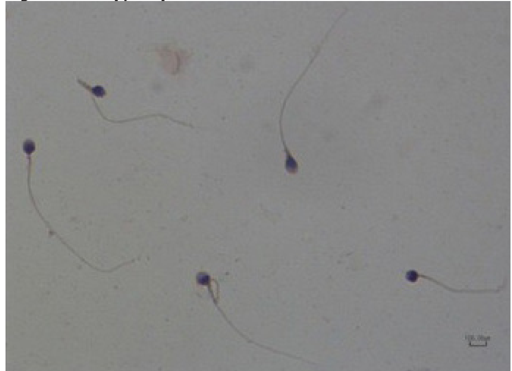

Figure 2. Evaluation of sperm morphology, representation of abnormal morphological structure of sperm

Table 1. Comparison of freezing solutions in healthy group

	average	standart deviation	p*
A solution /count after freezing	38,75	24,52	0,704
B solution/ count after freezing	37,15	22,10	0,704
C solution/ count after freezing	37,25	22,37	0,704
A solution / motile % after freezing	43,95	18,44	0,192
B solution / motile % after freezing	42,53	17,42	0,192
C solution / motile % after freezing	44,65	15,22	0,192
A solution/acridin orange DNA fragmantation after freezing	22,10	8,62	0,704
B solution/acridin orange DNA fragmantation after freezing	22,50	8,04	0,704
C solution/acridin orange DNA fragmantation after freezing	22,05	7,62	0,704
A solution aniline blue sperm maturation after freezing	23,00	7,95	0,818
B solution aniline blue sperm maturation after freezing	22,30	7,23	0,818
C solution aniline blue sperm maturation after freezing	23,60	7,70	0,818
there is no significant differences between in both group			

Table 2. Comparison of freezing solutions in patient group

	average	standart deviation	p*
A solution/count after freezing	2,15	1,30	0,321
B solution/count after freezing	2,12	1,34	0,321
C solution /count after freezing	2,50	1,67	0,321
A solution / motile % after freezing	36,75	10,42	0,786
B solution / motile % after freezing	34,30	14,60	0,786
C solution / motile % after freezing	36,10	12,24	0,786
A solution/acridin orange DNA fragmantation after freezing	44,30	10,45	0,860
B solution/acridin orange DNA fragmantation after freezing	44,70	9,47	0,860
C solution/acridin orange DNA fragmantation after freezing	44,80	10,84	0,860
A solution aniline blue sperm maturation after freezing	45,20	9,82	0,814
B solution aniline blue sperm maturation after freezing	46,05	10,31	0,814
C solution aniline blue sperm maturation after freezing	45,05	10,62	0,814
There is no significant differences between in both group			

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