Category: Clinical science

Topic: Embryology (incl. IVF - ICSI, oocyte and embryo selection, culture, cryopreservation, developmental biology, quality control)

Abstract title: ICM and TE quality significantly impact the live birth in euploid frozen blastocyst transfer cycles

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Study question: Is the live birth rate (LBR) in euploid frozen blastocyst transfer (FET) affected by the quality of ICM (Inner cell mass) and TE (Trophectoderm)?

Summary answer: ICM and TE significantly impacts the LBR with a decline of LB from 57.3% (ICM-A) to 48.5% (ICM-B) to 22.1% (ICM-C) ($p<0.001$)

What is known already: The morphological blastocyst grading system proposed by Gardner-Schoolcraft remains the most accepted system to identify blastocysts with higher implantation potential. It relies on morphological features within the blastocyst, including ICM and TE. Several studies tried to identify the individual contribution of each. However, the conclusions remain contradictory and no clear consensus has yet been achieved. Due to heterogeneity of parameters evaluated between different publications, where embryos with unknown ploidy status were transferred in conjunction with a variability of stimulation protocols and in the number of transferred embryos, the real effect of the ICM and TE is difficult to infer.

Study design, size, duration: This two-center retrospective observational study includes a total of 977 euploid single FET cycles between March 2017 and March 2020 at ART Fertility Clinics Muscat, Oman and Abu Dhabi, UAE.

Participants/materials, setting, methods: Trophectoderm biopsies were analyzed with Next Generation Sequencing (NGS). All blastocysts available on D5 or D6 with a quality ≥ BL3CC were subjected to TE biopsy for PGT-A analysis and LBR was recorded. Vitrification/warming of blastocysts was performed using Cryotop method (Kitazato). Bivariate and multivariate analysis were performed between LB outcomes and ICM and TE grade while controlling for confounding factors.

Main results and the role of chance: A total of 977 single FET cycles were analyzed: 651 in hormone replacement therapy (HRT) and 326 in natural cycle regimen (NC) resulting in a 46.88% LBR. The mean

patients' age was 33.80 years with a mean Body Mass Index (BMI) of 26.80 kg/m2. Though all qualities of ICM and TE were associated with LB, blastocyst ICM-A LBR was statistically significantly higher (57.3%) than ICM-B (48.4%) and ICM-C (22.1%) ($p<0.001$). Similarly, blastocyst TE-A LBR was statistically significantly higher (59.2%) than TE- B (48.6%) and TE- C (30.3%) ($p<0.001$). Miscarriage rate was similar in all groups.

The grade of ICM and TE were significantly associated with Anti-Mullerian-Hormone (AMH) and day of blastocyst biopsy. Mean AMH (ng/ml) was higher in ICM groups (A: 3.78, B: 3.24, $p<0.001$) and TE group (A: 3.63, B: 3.38, $p<0.05$) compared to lower grade (ICM-C: 2.86, TE-C: 2.82).

In multivariate analysis, endometrial preparation for FET, BMI and AMH were the parameters influencing LBR: OR:1.45, [CI:1.07-1.96], $p<0.015$) for NC; OR 0.96 [CI:0.93-0.99], $p=0.004$ for BMI; OR 0.95 [CI:0.90-1.00], $p=0.033$ for AMH; Both, ICM-C and TE-C, resulted in a significantly lower chance of LB [ICM: OR 0.32, CI:0.17-0.61, $p<0.001$; TE: OR 0.44, CI:0.27-0.73, $p=0.002$), compared to grade A.

Limitations, reasons for caution: The retrospective nature of the study and inter-observer variability in blastocyst scoring is a limitation. The physician/embryologist performing the embryo transfer could not been standardized due to the multicenter design. Randomized controlled studies are needed to determine whether ICM or TE should be prioritized in the selection of the blastocyst.

Wider implications of the findings: The ICM and TE scoring in FET may influence the LBR and should be considered as an important factor for the success of embryo transfer cycles. Whether these results can be extrapolated to fresh embryo transfer and to blastocysts with unknown ploidy status, needs further investigation.

Keywords: Inner cell mass, euploid frozen blastocyst transfer, trophectoderm, PGT-A, live birth

Category: Clinical science

Topic: Implantation and early pregnancy (incl. miscarriage, recurrent miscarriages, termination of pregnancy, ectopic pregnancy, pregnancy of unknown location, gestational trophoblastic disease)

Abstract title: Ongoing pregnancy rate (OPR) of day (D) 7 euploid blastocysts is inferior to D5/D6 euploid blastocysts in frozen embryo transfer (FET) cycles

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Study question: Do delayed-grown D7 euploid blastocysts have similar OPRs as D5 or D6 euploid blastocysts in FET cycles?

Summary answer: Although OPR is significantly higher with D5/D6 euploid blastocysts than D7, patients aged >38 years might benefit when slow-developing blastocysts are routinely cultured till D7.

What is known already: Current IVF practice suggests that embryos of optimal development reach blastocyst stage 116±2 hours after insemination. Recently, high reproductive potential has been reported with D6 as well as with D7 blastocysts. Although D7 blastocysts have a delayed embryo development, euploidy rates range between 25% and 49% if biopsy is performed. Usable D7 blastocysts represent nearly 5% of embryos in IVF with acceptable pregnancy and live birth rates, however, data are still limited. Therefore, further evidence of FET outcomes with D5, D6 and D7 euploid blastocysts are needed to investigate whether prolonged in-vitro embryo culture till D7 should be performed routinely.

Study design, size, duration: A single centre observational study was performed between June 2017 and November 2021, including 1396 single euploid FET cycles with blastocysts biopsied on D5 (N=795), D6 (N=572) or D7 (N=29). Patients underwent endometrial preparation for a FET in a natural cycle (NC) or hormone replacement treatment (HRT). Only blastocysts graded ≥ BL3CC (Gardner scoring) before trophectoderm (TE) biopsy on D5, D6 or D7, which re-expanded within 1-hour post-warming, were considered in the analysis.

Participants/materials, setting, methods: All warmed blastocysts were transferred after 120 hours of progesterone (P4) exposure. In NC, P4 was administered after ovulation until pregnancy test (PT). For HRT cycles, estradiol was prescribed until endometrial thickness reached ≥6 mm or a trilaminar pattern was seen on which P4 was supplemented until PT. OPR was recorded at 12 weeks by the presence of a gestational sac/s and fetal heartbeat. A multivariate logistic regression model with generalized estimating equation was performed.

Main results and the role of chance: Women's mean age differed significantly for FET cycles performed with D5, D6 and D7 euploid blastocysts (33.2±5.6, 34.5±5.3 and 36.1±4.5 years old; P< 0.001) as well as AMH values (ng/mL) (3.6±3.6, 2.9±2.8 and 2.3±1.8; P< 0.001; respectively). OPR with D5 euploid blastocysts was significantly higher than D6 and D7 (55.6%, 44.9% and 10.3%; P< 0.001), however, miscarriage rates did not differ (9.3%, 6.6% and 6.9%; P= 0.201; respectively). Following an adjusted multivariate logistic regression model, the factors associated with a reduced OPR were: D7 FETs (OR: 0.19 [0.06-0.63]; P= 0.006), ICM grade C (OR: 0.29 [0.17-0.48]; P< 0.001) and TE grade C (OR: 0.58 [0.38-0.89]; P= 0.012). Contrary, OPR outcomes were increased in NC compared to HRT cycles (OR: 1.35 [1.06-

1.71]; P= 0.013). A sub-analysis showed that advanced maternal age was a risk factor of having a D7 FET cycle (OR: 1.09 [1.01-1.17]; P= 0.025). In patients >38 years, OPR was improved if D7 FET cycles were performed however, this finding was nonsignificant (OR: 2.33 [0.19-29.4], P= 0.510). In patients <38 years, D7 FET cycles were significantly negatively associated with OPR outcomes (OR: 0.07 [0.01-0.54], P= 0.011). Regardless of patient's age, OPR outcomes with D5/D6 were higher than D7 FETs.

Limitations, reasons for caution: The current results are based on an observational study including a limited sample size of D7 euploid blastocysts FET cycles. Additionally, live birth rates should be considered in a further analysis to validate the performance of D5 and D6, compared to D7 euploid blastocysts, in both, NC and HRT cycles.

Wider implications of the findings: Considering age as a risk factor of having delayed grown blastocysts in-vitro, culturing embryos till D7 can be a strategy to increase OPR in patients >38 years old. With an increase in age, patients are more likely to have blastocysts biopsied on D6/D7. OPR outcomes are increased in NC FETs.

Keywords: Trophectoderm biopsy, PGT-A, Blastocyst culture, Euploid, Frozen embryo transfers

Category: Clinical science

Topic: Implantation and early pregnancy (incl. miscarriage, recurrent miscarriages, termination of pregnancy, ectopic pregnancy, pregnancy of unknown location, gestational trophoblastic disease)

Abstract title: Does C-Section history affect the live birth outcomes after IVF in case of frozen embryo transfers?

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Study question: Do previous Caesarean section deliveries (CD) affect reproductive outcomes in case of frozen embryo transfers (FET), including live births (LB), after IVF or ICSI?

Summary answer: Previous CD does not impair LBR after IVF when cycle segmentation is performed and intracavitary fluid (ICF) is eliminated prior frozen embryo transfer.

What is known already: Successful implantation requires not only a receptive endometrium, but also the process of 'apposition' and 'attachment' of the embryo to the endometrial surface. This process could be severely disturbed by previous CD and data are contradictory regarding its impact on ART outcome. Former CD might not only increase the difficulty of the ET-procedure, but might also lead to the presence of ICF. Therefore, special attention has to be paid to exclude fluid accumulation during preparation for FET. When ICF is excluded, the reproductive outcomes of FET cycles appear to be uncompromised, regardless of the previous delivery mode.

Study design, size, duration: This single center retrospective cohort study included a total of 412 single/double euploid FET cycles between March 2017 and October 2019. Trophectoderm biopsy samples were subjected to Next Generation Sequencing to diagnose the ploidy state. Vitrification and warming were performed using the Cryotop method (Kitazato, Biopharma). No embryo transfer was performed when ICF was visible during the endometrial preparation for FET.

Participants/materials, setting, methods: Patients with secondary infertility, having at least one euploid embryo after a previous IVF/ICSI-cycle with embryo vitrification, undergoing FET, were included. Clinical pregnancy rate (CPR), early pregnancy loss (EPL) and LBR were evaluated in patients after CD and after vaginal delivery (VD).

Main results and the role of chance: Patients in the CD group were significantly older than in the VD group (35.02±4.62 vs 34.11±5.03 years, respectively) (p=0.028). FET was performed in a Natural cycle (NC) (n=82, 46.86%) or in a Hormonal replacement therapy (HRT) cycle (n=93, 53.14%) in CD group versus NC (n=77, 32.49%) or HRT cycle (n=160, 67.51%) in VD group (p=0.003).

Patient characteristics (AMH, BMI, duration of infertility, presence of intrauterine abnormalities, endometrial thickness, embryo quality, presence of blood on the transfer catheter and number of SETs/DETs) were similar between groups. Embryo transfer difficulty and the presence of mucus on the transfer catheter were significantly higher in CD group versus VD group (p=0.040, p=0.004,

respectively). Pregnancy outcomes CPR (61.71% vs 63.71%), EPL (12% vs 12%) and LBR (60% vs 60.34%) did not differ statistically between the CD and VD groups.

In the multivariate regression model with embryo-quality, cycle-regimen, ET-difficulty, presence of blood/mucus, age, AMH, number of transferred embryos, only embryo quality remained significantly associated with LBR in CD-group (p=0.001). In VD-group, cycle regimen was also significant parameter besides embryo quality (p=0.001, p=0.001 respectively). When CD and VD groups are categorized in terms of cycle regimen, CP and LBR were similar (p=0.828/p=0.618 in HRT; p=0.826/p=0.150 in NC).

Limitations, reasons for caution: This study is limited to its retrospective design and the fact that no distinction existed between patients with one or several previous CDs. Furthermore, this analysis is limited to patients, for whom ICF was excluded prior to ET.

Wider implications of the findings: This study confirmed that in patients after CD, the chance for CP and LBR is not compromised, when ICF is excluded during the endometrial preparation for FET. The existence of C-section scar may increase the ET-difficulty and the presence of the mucus on the transfer catheter.

Keywords: C-Section History, Live Birth Rate, Frozen Embryo Transfer, PGT-A

Category: Clinical science

Topic: Reproductive endocrinology (incl. ovarian reserve testing, ovarian stimulation, IVM, POI, PCOS, infancy, disorders of sexual development, puberty, adolescence, menopause)

Abstract title: Reduction of gonadotropin-dosage towards the end of ovarian stimulation for IVF/ICSI improves ART-outcome in a subgroup of patients

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Study question: To identify in an unselected patient population those patients, who would benefit from a gonadotropin stepdown towards the end of ovarian stimulation in IVF/ICSI-treatment

Summary answer: Reduction of gonadotropin-dosage during ovarian stimulation for IVF/ICSI is beneficial in FSH-stimulated patients, aged 26-30 years, and in HMG-stimulated patients, aged 36-40 years of age

What is known already: Ovarian stimulation with gonadotropins for multi-follicular growth is a crucial part of IVF/ICSI-treatment. Intensive administration of gonadotropins might lead to progesterone elevation (PE) in the late follicular phase, impacting negatively the endometrial receptivity and possibly the embryo quality. During follicular growth, the number of FSH- and LH-receptors shift from predominantly FSH-receptors in the early to predominantly LH-receptors in the late follicular phase, reducing susceptibility of the follicle towards systemic FSH-levels. In a good prognosis population, it was shown that lower systemic FSH-levels on the trigger-day lead to significantly lower progesterone levels without reducing the number of retrieved/mature oocytes.

Study design, size, duration: Retrospective analysis of 1276 ovarian stimulation cycles, performed as GnRH (Gonadotropin-Releasing-Hormone)-antagonist protocol in an unselected patient population, between January 2018 and December 2020 at ART Fertility Clinic, Abu Dhabi, UAE. Ovarian stimulation cycles were split according to the stimulation medication used (either only recombinant FSH (rFSH) or only human menopausal gonadotropin (HP-HMG)) and whether stimulation-dosage was reduced (reduction group, RG) during the stimulation course or not (non-RG). Furthermore, patients were stratified into age-groups.

Participants/materials, setting, methods: Couples with primary / secondary infertility and an indication for ovarian stimulation for IVF/ICSI were included, independent of their age or ovarian reserve parameters. Only GnRH antagonist cycles, with either rFSH or HP-HMG as sole medication for ovarian stimulation and available data on Antral Follicle Count (AFC), gonadotropin-dosage at stimulation-start and -end, oocyte yield (number of retrieved / mature) and hormonal parameters of estradiol, progesterone and FSH were included.

Main results and the role of chance: A total of 1276 ovarian stimulation cycles were included, 495 (38.79%) with rFSH and 781 (61.21%) with HP-HMG as stimulation medication.

In the FSH-group, 295 patients reduced (reduction-group=RG) and 200 did not reduce (=non-RG) FSH-dosage during stimulation. In all age-categories (<25; 26-≤30; 31-≤35; 36-≤40; 41-45years), FSH-end-

dosage was significantly lower in the RGs compared to the non-RGs (p<0.001, respectively). Despite not significantly different patient characteristics (AFC, BMI (Body Mass Index), mean age, FSH-starting dosage), in patients aged 26-≤30years, a significantly higher number of oocytes (retrieved/mature) were obtained in the RG (16.0±7.1 vs 20.4±8.3;p<0.001 / 12.6±6.5 vs 15.8±7.0;p=0.004, respectively) without an increase in progesterone-levels (1.0±1.3 vs 0.9±0.6ng/ml;p=0.95).

The HP-HMG group was composed of 437 patients in the RG and 344 patients in the non-RG-group. In all age-categories (<25; 26-≤30; 31-≤35; 36-≤40; 41-52years), HP-HMG-end-dosage was significantly lower in the RGs (p<0.001, respectively). The age-group 36-≤40 showed no significant differences in AFC, BMI, age and HP-HMG-starting-dosage, and still, a significantly higher number of oocytes (retrieved/mature) were obtained in the RG-group (7.2±5.61 vs 9.8±6.13;p<0.001 / 5.7±4.7 vs 7.8±5.0;p<0.001, respectively). The progesterone-levels on trigger day in the nRG-group was 0.5±0.3ng/ml and 0.6±0.4ng/ml in the RG group, which is significantly different (p<0.001), however, clinically insignificant.

Limitations, reasons for caution: The retrospective design is a limitation to this study as well as the exclusion of ovarian stimulation cycles, which were cancelled and no oocyte pick-up procedure was performed. Furthermore, in some age-categories, patients in RG and non-RG were different regarding their basic characteristics.

Wider implications of the findings: This analysis demonstrates, that in specific patient populations, gonadotropin-dosage can be reduced without impacting the oocyte yield and preventing progesterone-elevation despite higher oocyte yield. Furthermore, reduction of gonadotropins towards the end of the follicular phase results in a more physiologic course of the gonadotropin-levels during ovarian stimulation.

Keywords: ovarian stimulation, reduction of gonadotropin dosage, progesterone elevation, oocyte yield

Category: Clinical science

Topic: Reproductive epidemiology, socio-cultural aspects and health economy (incl. cross border & developing countries)

Abstract title: PRIMARY SEX RATIO IS DECREASED IN EUPLOID EMBRYOS OF CONSANGUINE COUPLES AFTER IVF/ICSI WITH PGT-A

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Study question: What is the primary sex ratio outcome of embryos from consanguine couples as per PGT-A analysis during IVF/ICSI treatments?

Summary answer: The primary sex ratio (PSR) (males-to-females) is decreased in euploid blastocysts from consanguine couples.

What is known already: In the general population, the sex ratio (males-to-females) tends to be 1:1 (approximately 101 males to 100 females). In epidemiological studies secondary sex-ratio (ratio at the time of birth) of inbred-offspring seems to be equilibrated. However, it is well known that there is an increased incidence of autosomal recessive diseases for the offspring, compared to non-consanguineous couples. Studies have found elevated autosomal inheritances as compared to sex-linked inheritance. Assessing the impact of consanguinity on developed embryos might contribute to the understanding of the events leading to genetic diseases.

Study design, size, duration: This analysis includes data from 5135 blastocysts after preimplantation genetic testing for aneuploidy (PGT-A) with NGS. Embryos were obtained from 1836 IVF/ICSI cycles of infertile couples, at ART Fertility Clinics UAE, from November 2016 to December 2020. Consanguinity was defined when couple were first-degree or second-degree cousins.

Participants/materials, setting, methods: A total of 1138 blastocysts from consanguine couples, and 3997 from non-consanguine couples were included in the analysis. All blastocysts presented normal sexual chromosome constitution with or without autosomal aneuploidies. Mosaic and non-informative embryos were excluded. Trophectoderm biopsy was performed on day 5 for PGT-A using Next Generation Sequencing (NGS) platform. Primary sex ratio (PSR) was observed for CG and NCG couples. Ethical approval was obtained from the Research Ethics Committee (REFA023b).

Main results and the role of chance: In consanguine couples the age of female and male partner was 30.7±5.5 and 35.9 ±5.3 years old, respectively; while non consanguine couples were older (32.2±5.8 and 37.6±7.3 years old, respectively) ($p<0.001$).

Expanded blastocysts deriving from consanguine couples had 52.3% of XX versus 47.7% of XY constitution of their trophectoderm biopsied cells, presenting thus a significant decrease in primary sex ratio (PSR: 0.91, $p=0.03$). In non-consanguine couples, about 51.2% of trophectoderm biopsied cells had a XX constitution compared to 48.8% of XY constitution, presenting a PSR of 0.95 (NS).

The significant decreased PSR in consanguine couples was only related to normal euploid embryos and not to abnormal embryos (PSR of abnormal embryos = 0.98; NS). Euploid embryos from couples presenting consanguinity generated 53.4% of blastocysts of XX constitution versus 46.6% of XY constitution with a PSR at 0.87 (p=0.01).

Euploid embryos from non-consanguine couples presented a PSR at 0.96 (p=NS) (50.9% blastocysts of XX constitution versus 49.1% with XY constitution).

Limitations, reasons for caution: Differences in PSR between consanguine and non-consanguine couples did not reach significance level, justified by the lower number of embryos derived from consanguinity compared to non-consanguinity couples. Moreover 85% of the population included derives from Arabian Peninsula, therefore these results should not be transposed to other consanguine populations.

Wider implications of the findings: It is known that consanguine couples derive embryos with increased abnormalities in comparison to the general population. The fact that euploid embryos from consanguine couples seems to present a higher female constitution might incite us to investigate to what extent does heterozygosity for x-linked loci contribute to embryo survival.

Keywords: sex ratio, consanguinity, consanguine couples, PGT-A, Euploidy

Category: Clinical science

Topic: Reproductive endocrinology (incl. ovarian reserve testing, ovarian stimulation, IVM, POI, PCOS, infancy, disorders of sexual development, puberty, adolescence, menopause)

Abstract title: Natural endometrial preparation for single euploid frozen embryo transfer increases the likelihood of live birth in obese patients

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Study question: Does a natural endometrial preparation for single euploid frozen embryo transfer increase the odds of live birth (LB) in obese patients?

Summary answer: Compared to an artificial approach, a natural endometrial preparation for single euploid frozen embryo transfer improves the odds of LB in obese patients.

What is known already: Several protocols to prepare the endometrium for frozen embryo transfer have been described with comparable clinical outcomes, and no individual protocol has been demonstrated to be superior. Likewise, no specific patient group has been defined that can substantially benefit from a natural endometrial preparation. Despite obesity being linked to adverse clinical outcomes, observational studies and clinical trials have traditionally included participants with normal weight when comparing protocols, resulting in obese patients being underrepresented in clinical research. Therefore, studies focusing on endometrial preparation protocols for frozen embryo transfer that include obese patients are required.

Study design, size, duration: This study involved the analysis of a retrospective dataset including 975 single euploid frozen embryo transfer cycles performed at two tertiary referral centers between March 2017 and November 2019. The primary outcome was LB after natural and artificial endometrial preparation. Participants were stratified according to the World Health Organization Body mass index (BMI) classification into three groups (G): G1: Normal-weight ≤24.9 (n=390), G2: Overweight 25 to 29.9 (n=332), and G3: Obese ≥30 (n=253).

Participants/materials, setting, methods: Cycles included infertility patients attempting a single euploid frozen blastocyst transfer under natural (n=324) or artificial (n=651) endometrial preparation. PGT-A was performed after trophectoderm biopsy using Next-Generation Sequencing. Spontaneous LH rise confirmed ovulation for natural endometrial preparation, and embryo transfer occurred five days after initial progesterone elevation. Hormones were measured from blood samples. For artificial preparation, embryo transfer was performed after oral estradiol and 120-hour vaginal progesterone exposure. All participants received luteal phase support.

Main results and the role of chance: Within each BMI group, no statistically significant differences in age were observed in patients between a natural and artificial endometrial preparation: G1 (33.5±5.1 vs. 32.7±5.2 years; p=0.13), G2 (34.5±4.9 vs. 33.9±5.8 years; p=0.39), and G3 (35.6±4.9 vs. 34.3±5.6 years;

p=0.1). Similarly, no statistically significant differences were observed regarding implantation rates: G1 (60% vs 57.1%; p=0.57), G2 (54.8% vs 55.7%; p=0.88), and G3 (64.3% vs 51.4%; p=0.07). LB rates remained constant across all BMI groups when a natural preparation was conducted: G1 [56.66% (n=85/150)], G2 [49.03% (n=51/104)], and G3 [58.57% (n=41/70)]. We observed a decrease in LB rates in G3 when an artificial endometrial preparation was performed: G1 [47.5% (n=114/240)], G2 [44.73% (n=102/228)], and G3 [34.42% (n=63/183)]. The positive impact of a natural preparation on LB rates was more significant in G3, showing a 24% difference of effect between the natural and artificial endometrial preparations. Univariate logistic regression analysis showed a statistically significant difference in the primary outcome (LB) when a natural endometrial preparation was conducted in G3 (OR 2.69, 95% CI 1.53-4.74; p=0.001), with no differences found in G1 (OR 1.45, 95% CI 0.96-2.18; p=0.08) and G2 (OR 1.19, 95% CI 0.75-1.89; p=0.73).

Limitations, reasons for caution: This study could be subject to bias due to its retrospective nature. Sperm quality was not considered for the present study.

Wider implications of the findings: Obese women appear to be the patient group that can obtain benefit from a natural endometrial preparation for frozen embryo transfer by increasing the odds of LB. Future prospective studies should confirm this hypothesis. A natural approach might be unfeasible in a subgroup of obese patients due to anovulatory cycles.

COI: I have no potential conflict of interest to disclose

Keywords: Live birth; Natural cycle; Artificial cycle; Obesity; Endometrial preparation

Category: Clinical science

Topic: Embryology (incl. IVF - ICSI, oocyte and embryo selection, culture, cryopreservation, developmental biology, quality control)

Abstract title: Identifying patients benefiting from delayed-matured oocytes insemination

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Study question: Are there any predictive factors supporting the decision of inseminating delayed-matured oocytes?

Summary answer: Patients with ≤59% mature oocytes at retrieval and/or Anti-Mullerian-Hormone (AMH) >2.52 ng/ml have increased chances of obtaining a euploid embryo from delayed-matured oocytes.

What is known already: Approximately 15% of oocytes retrieved after ovarian stimulation are immature, at metaphase I (MI) or germinal-vesicle (GV) stages at the time of oocyte denudation. Performing IVM in those oocytes could permit an increase on the number of usable embryos. Nevertheless, the utility of delayed-matured oocytes varies greatly among IVF laboratories with relatively low success rates, hence its practice in daily routine might be counter-productive. Determining which patient population could benefit from such strategy is valuable thereof to the clinical practice. Moreover, data comparing euploid rates of embryos derived from delayed-matured oocytes with its mature sibling oocytes are needed.

Study design, size, duration: This observational study was performed at ART Fertility Clinics, Abu Dhabi, UAE, between January 2019 and June 2021. A total of 5454 cumulus oocytes complexes (COC) were retrieved from 469 ovarian stimulation cycles. Out of the retrieved COCs, 3473 oocytes were immediate at metaphase II (MII-D0), and 915 were delayed-metaphase II oocytes (MII-D1).

Participants/materials, setting, methods: Patients with primary and secondary infertility undergoing Controlled ovarian stimulation (COS) in standardized protocols for IVF/ICSI treatment were included. Ovum pick up performed 34-36h post final oocyte maturation trigger shot (TS). Insemination was done 39-41h post TS for the MII-D0, while MII-D1 ICSI was performed 63-68h post TS. All cycles were planned for Preimplantation Genetic Testing for Aneuploidies (PGT-A) at blastocyst stage using Next Generation Sequencing (NGS).

Main results and the role of chance: Fertilization rates significantly differed between MII-D0 and MII-D1 oocytes (69.54% vs 55.96%, $p<0.001$, respectively). Blastocyst utilization rates were significantly higher in MII-D0 group compared to MII-D1 group (59.47% vs 18.52%, $p<0.001$). However, no difference was observed in the rate of euploid blastocysts between MII-D0 and MII-D1 (46.3% vs 39.0%, $p=0.163$).

As identified by univariant logistic regression analysis, the following parameters augmented the chances of obtaining at least 1 blastocyst for biopsy when MII-D1 were injected: AMH (OR 1.15, p<0.001), number of COCs collected (OR: 1.03, P=0.005), maturation rate on day0 (OR: 0.19, P=0.001).

When the multivariant analysis model was applied, AMH and maturation rate on day0 remained significant factors predicting the success of inseminating delayed-matured oocytes (OR:1.15, [CI:1.00-1.32], p=0.045); OR:0.06, [CI:0.03-0.31], p<0.001, respectively), with cut off values of AMH >2.52 ng/ml and maturity rate of ≤59%, being identified by ROC analysis.

Limitations, reasons for caution: ICSI of MII-D1 was performed with the fresh or frozen sperm samples from the previous day. Exact timing of polar body extrusion of delayed-matured MI/GV was not identified.

Wider implications of the findings: The results of this study might provide guidance to the IVF laboratories for targeting the patient population who would benefit from MII-D1 ICSI without adhering to unnecessary costs and workload.

Keywords: Delayed-Matured oocytes, IVM, Rescue IVM, Euploidy, immature oocytes

Category: Clinical science

Topic: Reproductive endocrinology (incl. ovarian reserve testing, ovarian stimulation, IVM, POI, PCOS, infancy, disorders of sexual development, puberty, adolescence, menopause)

Abstract title: Do ovarian reserve markers and female age predict the rate of euploid blastocysts in IVF/ICSI cycles?

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Study question: Are female age and ovarian reserve markers (anti-Mullerian hormone (AMH) and Antral Follicle Count (AFC)), able to predict euploid blastocyst rate in IVF/ICSI cycles?

Summary answer: Female age, serum AMH, AFC and the number of mature oocytes collected during IVF/ICSI cycles significantly affect the rate of euploid blastocysts

What is known already: The age-associated decline in female reproduction has been clearly demonstrated, due to the reduction of the ovarian reserve and the increased risk of chromosomal abnormality occurring in the oocyte. Lately, it has been debated whether a reduced ovarian reserve, independently of age, could be associated with higher aneuploidy rate in embryos. Ovarian reserve can now be accurately measured by serum AMH levels and AFC, both markers with similar high reliability.

Study design, size, duration: This analysis includes data from 10556 blastocysts after preimplantation genetic testing for aneuploidy (PGT-A) with Next Generation Sequencing (NGS). Embryos were obtained from 2564 IVF/ICSI cycles of infertile couples, at ART Fertility Clinics UAE, from November 2016 to December 2020.

Participants/materials, setting, methods: 10556 blastocysts with chromosomal information for ploidy were included, mosaic and non-informative embryos were excluded. Trophectoderm biopsy was performed on day 5, 6 or 7 blastocysts. Serum AMH concentrations were measured by Elecsys® AMH automated assay (Cobas 601 platform, Roche®) for all patients in a single laboratory. AFC (sum of small antral follicles in both ovaries) was evaluated with transvaginal 2D-sonography (Voluson E8, GE Healthcare). Ethical approval was obtained from the Research Ethics Committee (REFA023b).

Main results and the role of chance: Patients' characteristics are described as mean ± SD (min-max): age: 34.72±6.13(18-50), years of infertility: 3.43±3.43(0-25), AMH: 2.52±2.70ng/mL(0.01-23.00), AFC: 11.57±7.86(0-61), body mass index (BMI): 28.57±4.83Kg/m2(14.34-44.96), Metaphase II (MII) inseminated oocytes: 10.11±6.53(1-50), 2PN embryos 7.32±5.12(1-42), blastocysts 4.12±3.21(1-26). Fertilization rate was 73.31%(±19.30), blastulation rate 61.05%(±25.69) and euploidy rate 39.42%(±35.24).

A significant negative Pearson correlation coefficient was found between age and euploidy rate (ρ=-0.5398, p<0.001). AMH, AFC and total of MII inseminated oocytes showed a significant positive Pearson correlation coefficient with euploid rate (AMH:ρ=0.2076, p<0.001; AFC: ρ=0.2578, p<0.001; MII:ρ=0.2036, p<0.001). Linear regression analysis was conducted to evaluate the predictability of the variables on euploid rate. As expected, age clearly had a negative impact (Coef=-3.10, Std. Err=0.10, p<0.0001). A positive effect was observed for AMH (Coef=2.75, Std. Err=0.31, p<0.0001), AFC (Coef=1.16, Std. Err=0.09, p<0.0001), number of MII inseminated oocytes (Coef=1.10, Std. Err=0.10, p<0.0001) and 2PN embryos (Coef=1.43, Std. Err=0.13, p<0.0001). For patients >35 years old and AMH lower than 1.3 ng/mL, euploid rate was significantly lower compared with the patients >35 years old and AMH equal or higher than 1.3 ng/mL (21.2% vs 25.5%, p=0.0192).

Limitations, reasons for caution: Despite the large number of cycles and embryos included, the retrospective study design is a limitation.

Wider implications of the findings: Ovarian reserve is not only a quantitative, but also a qualitative biomarker of oocyte-embryo competence. Cumulative success rates for IVF/ICSI cycles are dependent on the availability of euploid blastocysts. Age and ovarian reserve markers should be combined for adequate counselling.

Keywords: AMH, Antral follicle count, female age, euploid blastocyst, IVF/ICSI

Category: Clinical science

Topic: Reproductive endocrinology (incl. ovarian reserve testing, ovarian stimulation, IVM, POI, PCOS, infancy, disorders of sexual development, puberty, adolescence, menopause)

Abstract title: Impact of systemic FSH levels on the embryo ploidy status in ovarian stimulation for IVF/ICSI

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Study question: Does systemic serum FSH-level per mature oocyte (FSH/MII) on the day of final oocyte maturation affect the ploidy status of the embryo cohort?

Summary answer: Higher serum FSH/MII levels negatively affect the euploid rate of the embryo cohort.

What is known already: In ovarian stimulation cycles, follicular phase systemic FSH-levels have to be kept above a certain threshold to achieve and maintain multifollicular growth. This contrasts the natural pattern, where FSH-levels decline steadily in the later follicular phase as a result of the increasing estrogen level. Until now, it is unclear, whether this unphysiological pattern of the systemic FSH course has an influence on the ploidy status of the biopsied blastocysts.

Study design, size, duration: Retrospective study performed at a tertiary ART referral center, including 582 cycles between March 2017 and December 2020. All GnRH (Gonadotropin-Releasing-Hormone) antagonist stimulation cycles used only recombinant FSH as gonadotropin. Patients aged between 19 and 48. All embryos underwent Preimplantation Genetic Testing for Aneuploidies (PGT-A) by Next Generation Sequencing with trophectoderm biopsy. Patients with surgical sperm extraction or warmed oocytes were excluded.

Participants/materials, setting, methods: Patients with primary or secondary infertility and an indication for ovarian stimulation for IVF/ICSI with PGT-A were included. Ovarian stimulation cycles were monitored according to clinical routine by ultrasound and repeated measurement of FSH, estradiol (E2), progesterone (P4) and LH throughout the cycle. The FSH/MII ratio was calculated as the ratio of the systemic FSH-level on the day of trigger (DoT) to the total number of mature oocytes (MII) after denudation.

Main results and the role of chance: FSH-levels at DoT revealed a wide range of systemic levels, from 2.12 to 47 IU/L. Patients had a mean age (±SD) of 30.49±4.99 years, Body Mass Index (BMI) of 27.58±5.14 kg/m2, Anti-Müllerian Hormone (AMH) of 4.20±3.05 ng/ml and 17.54±8.63 oocytes were collected with the oocyte pick up procedure.

In the univariate analysis, a significant negative correlation was found between euploid rate and the FSH/MII ratio (β=-3.194, $p<0.001$). Other parameters found to be negatively correlated were age (β=-1.055, $p<0.001$), systemic FSH-level at DoT (β=-0.652, $p<0.01$), total stimulation dose (β=-0.477, $p<0.01$) and basal FSH-level (β=-1.605, $p<0.05$). Alternatively, basal antral follicle count (AFC; β=0.416, $p<0.05$)

and total follicle count at DoT (β=0.507, p<0.01) were observed to have a positive significant correlation with the euploid rate.

After adjusting for potential confounders in a multivariate analysis, three parameters were still observed to be significantly correlated: FSH/MII ratio remained a negative factor for euploid rate (β=-2.753, p<0.01) besides the parameters age (β=-0.837, p<0.01) and E2 at DoT (β=-0.439, p<0.05).

Limitations, reasons for caution: The retrospective character of this study can be seen as a limitation as well as the fact that the results cannot be translated to patients using either only HMG, or a combination of recombinant FSH and HMG for ovarian stimulation.

Wider implications of the findings: Due to the fact that higher FSH/MII ratios are associated with a decreased euploid rate, FSH-level measurements should be introduced into ovarian stimulation monitoring, as the gonadotropin dosage might be adjusted according to the systemic FSH-levels. This represents a further step on the treatment individualization towards a more personalized medicine.

Keywords: FSH-Level, Day of Trigger, Euploid Rate, Ovarian Stimulation, FSH/MII ratio

Category: Clinical science

Topic: Implantation and early pregnancy (incl. miscarriage, recurrent miscarriages, termination of pregnancy, ectopic pregnancy, pregnancy of unknown location, gestational trophoblastic disease)

Abstract title: Clinical and laboratory factors associated with pregnancy outcomes in patients undergoing frozen euploid blastocyst transfer

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Study question: Which variables do have an impact on the pregnancy and live birth rates (LBR) when euploid frozen embryo transfers (FET) are performed?

Summary answer: Day of trophectoderm biopsy, Body Mass Index (BMI) and endometrial preparation protocol have an impact on pregnancy rate (PR) and LBR in FET cycles.

What is known already: Preimplantation genetic testing for aneuploidy (PGT-A) and morphological grading of embryos are the two main criteria to select a blastocyst from a pool of embryos, having the highest implantation potential. However, other clinical and laboratory variables might play a crucial role for a successful outcome when top quality euploid embryos are transferred in a FET cycle. It has been described that higher BMI increases the odds for miscarriage when compared with non-obese women.

Study design, size, duration: This analysis includes 1660 FET cycles with data from of pregnancy rates, miscarriage rate and LBR. Embryos were obtained from 2564 IVF/ICSI cycles of couples with primary or secondary infertility at ART Fertility Clinics UAE, from November 2016 to December 2020.

Participants/materials, setting, methods: A total of 1660 FET cycles with 2439 euploid blastocysts were included. FET cycles with mosaic or segmental aneuploid embryos were excluded. PGT-A was performed on trophectoderm cells, using Next Generation Sequencing (NGS). Biopsy was performed on day 5, 6 or 7 blastocysts. Endometrial preparation included ovulatory natural cycles (NC) and hormone replacement therapy (HRT) cycles, chosen according to physician's discretion. Ethical approval was obtained from the Research Ethics Committee (REFA023b).

Main results and the role of chance: Patients' characteristics are described as mean±SD (min-max): age: 33.5±5.43(19-47), AMH: 3.30±3.05ng/mL(0.01-23.00), BMI: 27.1±4.87Kg/m2(13.1-43.90), mean number of blastocysts transferred: 1.4±0.49(1-3). Patients were categorized according to age (years) in 4 categories (<30,n=404; 30-34,n=487; 35-40,n=595; >40,n=174) and no association with pregnancy rate (PR, p=0.856), biochemical miscarriage rate (BMR, p=0.940), clinical miscarriage rate (CMR, p=0.06) nor LBR (p=0.154) was found. BMI (kg/m2) was divided into four groups according to World Health Organization: underweight (<18.5;n=32), normal weight (18.5–24.9;n=555), overweight (25–

29.9;n=622), and obese (≥30;n=426). Although no differences were seen for PR or BMR between groups (p=0.507 and p=0.343, respectively), CMR was significantly lower for normal BMI group (p<0.001) and LBR significantly higher when compared to the overweight and obese group (<18.5kg/m2=68.42%; 18.5–24.9kg/m2=68.35%; 25–29.9kg/m2=60.14%; ≥30kg/m2=53.29%; p<0.001). No differences were observed on the outcomes when AMH was sub-divided as per Bologna Criteria (<1.3ng/mL,n=327; ≥1.3ng/mL,n=1090). Regarding endometrial preparation, NC protocol showed significantly lower BMR and CMR (7.93% vs 12.27%,p=0.026; 8.44% vs 17.97%,p<0.001), and higher LBR (70.33% vs 55.06%,p<0.001) compared to HRT. Day of trophectoderm biopsy had a significant higher PR for day 5 (day 5=75.58% vs day 6=61.1% and day 7=23.81%, p<0.0001), yet no differences were observed for BMR, CMR nor LBR.

Limitations, reasons for caution: Although the large number of FET included, performed in the same centre with same methodology, the retrospective study design is a limitation. We could not discard other hypothetical variables contributing to miscarriage such as KIR-HLA discrepancies, or other obstetric factors affecting late miscarriage and live birth.

Wider implications of the findings: Evaluating the factors associated with pregnancy outcomes should be considered prior to euploid frozen embryo transfer for personalized treatment approach and adequate blastocyst selection. Women with higher BMI should be aware of higher risk of miscarriage and lower LBR although an euploid blastocyst is transferred.

Keywords: Frozen embryo transfer, Endometrial preparation, body index mass, trophectoderm biopsy, PGT-A

Category: Clinical science

Topic: Implantation and early pregnancy (incl. miscarriage, recurrent miscarriages, termination of pregnancy, ectopic pregnancy, pregnancy of unknown location, gestational trophoblastic disease)

Abstract title: Effect of endometrial thickness on biochemical pregnancy rate: an analysis of 1534 frozen euploid embryo transfers

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Study question: Can a thick endometrial lining measured prior to embryo transfer be considered a protective factor against Biochemical Pregnancy (BP)?

Summary answer: The risk of BP is independent of Endometrial Thickness (EMT), but rather dependent of the type of endometrial preparation and parity.

What is known already: Higher EMT prior to embryo transfer is associated with better clinical outcomes in general, like higher implantation and livebirth, and lower miscarriage rates. But up to our knowledge, no studies evaluated the effect of EMT on BP per say.

Study design, size, duration: This is a two-center retrospective observational study including a total of 1534 euploid Frozen Embryo Transfer (FET) cycles between March 2017 and March 2020 at ART Fertility Clinics Muscat, Oman and Abu Dhabi, UAE. BP is defined as blood beta-hCG >15 mIU/ml on day 12 post FET, that is progressively decreasing, with no evidence of gestational sac on ultrasound.

Participants/materials, setting, methods: The study group consisted of 112 cases of BP, while the control group consisted of the remaining 1422 FET's that led to different clinical outcomes. EMT was measured by transvaginal ultrasound on the day of progesterone rise (±1 day); that rise was either spontaneous in Natural Cycles (NC), or iatrogenic in Hormone Replacement Therapy (HRT) cycles. Euploidy status of the embryos was assessed by NGS analysis of trophectoderm biopsies. Bivariate and multivariate analyses were conducted.

Main results and the role of chance: There was no difference in mean EMT between the study and the control groups (7.55 vs. 7.68 mm, p=0.154).

Looking at the association of different variables with the rate of BP, there was no effect of age, BMI, AMH, number of embryos transferred, degree of blastocyst expansion, inner cell mass or trophectoderm grade, day of biopsy, nor presence of blood or mucus on the transfer catheter. However, patients on HRT cycles had significantly higher rates of BP compared to NC (8.42% vs. 4.99%, p=0.015). Also, those with a previous livebirth had higher rates of BP compared to nulliparous women (8.7% vs. 5.39%, p=0.014).

The distribution of BP showed that 54.5% occurred with EMT <7.5 mm, 34.8% with EMT 7.5-9 mm, and 10.7% with EMT >9 mm. These represents respectively 8.16%, 6.68%, and 5.94% of the total sample. This decreasing trend of BP with increasing EMT didn't reach statistical significance (p=0.429). Univariate analysis comparing the risk of BP in FET's done with lower and higher EMT to those performed at 7.5-9 mm yielded similar conclusion: OR=1.24 [0.82-1.88] for <7.5 mm, and OR=0.88 [0.45-1.72] for >9 mm.

Controlling for different confounders, HRT cycles and multiparity remained as independent risk factors for BP.

Limitations, reasons for caution: Inter-observer variability in EMT measurement and the transfer technique, the retrospective nature of the study, and the lack of data on the mode of delivery of parous women could all have interfered with the conclusion.

Wider implications of the findings: The reduced adverse clinical outcomes with NC shed light on the role of the corpus luteum in the early phases of implantation, and some potential secreted mediators other than progesterone. Besides, the effect of previous deliveries on the endometrium and its receptivity needs further investigation.

Keywords: Biochemical pregnancy, Endometrial thickness, Frozen embryo transfer, Natural cycle, Endometrial preparation