cycle genes, CENPE, AURKA, BUB1 and MAD2L1 were selected for detailed molecular analysis for Poly(A) tail lengths. Poly(A) transcripts were G-tailed, reverse transcribed using a $C_{12}T_6$ primer and then amplified in two rounds using nested gene specific primers close to the 3′ end in combination with the $C_{12}T_6$ primer. PCR products were purified and cloned into a TA vector and transfected into E. coli. Inserts were individually selected and sequenced to determine the exact number of A nucleotides in the Poly(A) tail.

RESULTS: For all genes examined the Poly(A) tail length was significantly longer in the IVM oocytes compared to MII oocytes ($P<0.01$). Additionally, with the exception of AURKA, all genes examined had a significantly longer Poly(A) tail length in IVM oocytes compared to the GV oocytes from which these were derived ($P<0.001$).

CONCLUSIONS: For the cell cycle genes examined, IVM oocytes have a significantly longer Poly(A) tail than MII oocytes indicating dysregulation of the normal mRNA processing that occurs during maturation in vivo. Failure to deadenylate or precocious polyadenylation of genes in IVM oocytes may explain the basis of the developmental incompetence of these oocytes.

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THE ZONA PELLUCIDA BIREFRINGENCE IN IMMATURE AND MATURE OOCYTES COLLECTED AFTER CONTROLLED OVARIAN STIMULATION: PRELIMINARY RESULTS. D. P. A. F Braga, P. Queiroz, R. d. C. S. Figueira, L. G. L. Maldonado, A. Iaconelli, Jr., E. Borges, Jr. Scientific Research, Fertility - Assisted Fertilization Center, São Paulo, Brazil; Andrology Laboratory, Fertility - Assisted Fertilization Center, São Paulo, Brazil; IVF - Laboratory, Fertility - Assisted Fertilization Center, São Paulo, Brazil; Clinical Department, Fertility - Assisted Fertilization Center, São Paulo, Brazil.

OBJECTIVE: The zona pellucida (ZP) is a dynamic matrix composed of filaments organized in layers that differs in their orientation. Different development stages and culture conditions may alter the ZP architecture. The introduction of polarization light microscopy allowed for the non-invasive visualization of subcellular structures in oocytes, such as the ZP. It was demonstrated that birefringence of ZP (ZB) changes with different maturational stages of the oocyte. We evaluated the ZB in immature and mature oocytes collected after controlled ovarian stimulation and assessed the influence of ZB on *in vitro* maturation (IVM), fertilization and embryo quality.

DESIGN: Pilot study.

MATERIALS AND METHODS: It was evaluated 99 oocytes: 72 at metaphase II-stage (MII), 10 at metaphase I-stage (MI) and 17 at prophase I-stage(PI), collected from 12 patients (36.0 ± 3.2-yr-old) undergoing ICSI cycles. After cumulus removal, oocytes were imaged though a polarization system prior to ICSI for MII oocytes or prior to IVM for MI and PI oocytes. Oocytes were classified as having either high (HZB) or low birefringence (LZB) of the ZP. The ZB was compared among MII, MI and PI oocytes and to study the influence of ZB on IVM, fertilization and embryo quality a regression model was conducted.

RESULTS: The number of HZB oocytes was higher in immature when compared to MII oocytes (62.9% vs 12.5% $P<0.001$). Among the immature oocytes it was also observed an increased number of HZB in PI oocytes (82.4% vs 30%, for PI and MI oocytes respectively, $P=0.007$). *In vitro* maturation was achieved by 60% of the MI and 23.5% of the PI oocytes ($P=0.058$). Although it did not reach statistical significance, trends toward a positive influence of the ZB on IVM (OR = 1.63 IC 95% = 0.31 – 8.61; $p = 0.559$), fertilization (OR = 1.23 IC 95% = 0.19 – 3.15; $p = 0.716$) and embryo quality (OR = 1.33 IC 95% = 0.27 – 6.58; $p = 0.723$) was noted.

CONCLUSIONS: The ZP plays important roles during oogenesis, fertilization and embryos development. The exact stage at which the human ZP appears and whether this structure alters during development is unclear. We observed that ZB decreases as oocyte nuclear maturation takes place. Indeed, it was previously suggested that the ZP protein levels diminishes during oogenesis. In addition, we observed that ZB may predict IVM, fertilization and embryo quality, however probably because of the small number of oocytes evaluated during this trial it was not statically significant. This study is to be continued to confirm our findings.

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EFFECT OF ISOBUTYLMETHYLXANTHINE (IBMX) ON CHROMATIN CONFIGURATION AND MORPHOLOGY OF HUMAN GERMINAL VESICLE (GV) OOCYTES. L. Escrich, N. Grau, E. García-Roselló, A. Pellicer, M.-J. Escribá. Universitary Institute IVI Valencia, Valencia, Spain.

OBJECTIVE: IBMX inhibits meiosis and improves competence of in vitro matured (IVM) oocytes. Morphologically, its effect was never described in humans. Here, we studied the effect of IBMX, on chromatin configuration (CC) and morphology of human GV.

DESIGN: Experimental research.

MATERIALS AND METHODS: A total of 114 GV from stimulated cycles were donated. Sixty-one GV were incubated in 50mg/ml IBMX for 1-16 hours and 53 ova not (controls). Under contrast-phase microscopy, GV were assessed morphologically for size, nuclear and nucleolar diameters. Continuity of the nuclear envelop (NE) was also assessed and scored from grade 1 (continuous) to grade 4 (unidentifiable). Then, within every experimental group, CC was determined by Hoescht and fluorescent microscopy. ANOVA test was applied.

RESULTS: After morphologic and fluorescent evaluation, IBMX and control GV were assigned to one out of four earlier reported CC groups.

TABLE 1. Frecuency distribution and morphometrics of control and pretreated IBMX GV oocytes, according to four CC categories

		Number of ova (%) at the CC of:	Diameter of (μm):		
			Ova	GV	Nucleolus
Control	GV1	17 (32.0)	102.74±10.17	29.34±2.42ac	9.08±1.63
Control	GV2	5 (9.4)*	104.31±4.15	32.20±0.95a	9.31±1.64
Control	GV3	26 (49.0)**	103.64±5.40	28.81±2.41ac	8.68±1.16
Control	GV4	5 (9.4)	100.10±8.14	23.36±6.49b	6.55±1.19
IBMX	GV1	16 (26.2)	102.83±6.80	28.30±3.37c	8.50±2.03
IBMX	GV2	26 (42.6)*	104.59±5.14	28.73±1.73c	7.99±1.79
IBMX	GV3	12 (19.6)**	103.58±4.72	28.52±1.23c	8.46±0.95
IBMX	GV4	7 (11.4)	102.58 ±4.97	27.75±3.04c	7.24±1.55

*·** <0.05; Different superscripts within a column indicate significant differences.

Significantly more ova were at GV2 after IBMX; controls were usually at GV3. Concerning morphometrics, differences were observed in the nuclear size of controls ($P=0.001$), being GV4 the smallest. These differences were not detected in IBMX group; measure of ova and nucleolus were comparable, regardless experimental group and CC (102.65μm and 8.15μm in average). In IBMX group, CC was not related to NE discontinuity ($p=0.693$), unlike controls ($p=0.003$).

CONCLUSIONS: IBMX could likely arrest oocytes at GV2, affecting CC and subcellular structures. Three evidences from IBMX group support that; (1) observed alteration on GV frequency distribution according to CC, (2) nuclear size comparable to GV2 controls and (3) blockage on regular NE progression to disorganization.

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SUCCESSFUL SPONTANEOUS IN VITRO MATURATION OF HUMAN GERMINAL VESICLE (GV) OOCYTES, RECOVERED FROM STIMULATED CYCLES. M. J. Escriba, N. Grau, L. Escrich, E. García-Roselló, A. Pellicer. University Institute IVI Valencia, Valencia, Spain.

OBJECTIVE: After controlled ovarian hyperstimulation, most of recovered ova are mature at the metaphase II (MII: 85%) while others immature, being at either the metaphase I (MI: 4%) or the GV stage (11%). To study the nuclear and cytoplasmic competence of GV oocytes was aimed here.

DESIGN: Experimental research, approved by the Ethical Comitee of the Instituto Universitario IVI, Law14/2003, Spain.

MATERIALS AND METHODS: A total of 159 GV oocytes from stimulated cycles were donated to this research. Individual GV oocytes were matured in 50μL hTF medium for 2 days. Progression on nuclear maturation was assessed twice along the in vitro maturation (IVM) period; at IVM_1