MATERIALS AND METHODS: We performed ovarian hyperstimulation on 8-week-old female BDF1 mice by administering eCG 7.5 IU or hCG 7.5 IU. After rearing these females in the same cage with males of the BDF1, fertilized eggs were collected from oviducts of the plug-positive females. Plastic dishes (Falcon) with a 35-mm aperture were prepared with five 20-μL drops of M16, covered with mineral oil (Vitrolife), placed in a 60-mm dish (Falcon), and then cultured at rest in an incubator (HC-3100 ASTEC). Five to ten embryos were cultured per drop. Labels using acrylic adhesive were affixed to the inside of the lid of the 60-mm dish, and culturing was performed at 37°C, 5% CO_2, 5% O_2, 90% N_2, and saturated humidity. The number of affixed labels totaled 0 (control group), 1 (group A), 7 (group B), or 13 (group C), and development of the cultured mouse embryos from the 1-cell stage was investigated for 96 hours. Statistically analysis between experimental groups were determined by one-way ANOVA followed by Tukey test for multiple comparisons. Following the approval by the Animal Care and Use Committee of Azabu University, we used 263 mouse embryos in this study, which was performed at the Azabu University in the duration from September 2015 to December 2015.

RESULTS: The cleavage rate of the control group and groups A, B, and C were 97.5%, 97.1%, 12.0%, and 9.5%, respectively, with significant ($P<0.001$) decreases in groups B and C compared with the control; the developmental rate to the blastocyst stage were 88.6%, 0%, 0%, and 0%. As increasing the number of label, the incidences of cleavage of the cultured embryos were reduced, and there was no embryo developed to the blastocyst stage in all of the label-affixed groups.

CONCLUSIONS: Affixing labels using acrylic adhesive on culture dishes have detrimental effects on the development of mouse embryos at 1-cell stage. It is concluded that careful consideration should be given to the adhesive's components when using label stickers for culture dish management.

References:

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WASHING MINERAL OIL USED FOR MICRODROP OVERLAY DOES NOT IMPROVE STABILITY OF MEDIA OSMOLALITY. J. E. Swain,[a] A. E. Batcheller,[b] W. B. Schoolcraft,[c] N. Bossert.[b] [a]CCRM IVF Network, Lone Tree, CO; [b]CCRM Minneapolis, Edina, MN; [c]Medical Director, Colorado Center for Reproductive Medicine, Lone Tree, CO.

OBJECTIVE: Evaporation of culture media and the resulting increase in osmolality is a concern in some IVF laboratories, especially considering the increased use of non-humidified benchtop incubators. This concern may be increased when using single-step embryo culture media and uninterrupted culture over periods of up to 7 days. Some conjecture that washing of oil "saturates" the oil and prevents absorption of media components from microdrops, thereby possibly protecting growth conditions. The impact of extended uninterrupted culture and washing of mineral oil used for overlay on osmolality of microdrops was examined.

DESIGN: Basic research study.

MATERIALS AND METHODS: Microdrops of media (25ul) were prepared in a laminar flow hood at room temperature by pipetting 12.5μl of media onto the surface of a 35mm dish, covering with 3.5ml of unwashed or washed mineral oil (Ovoil, Vitrolife), removing the media and adding 25μl of fresh media. Washed oil was prepared by adding culture media in a 1:5 ratio to the oil, inverting 30 times and allowing to settle. Dishes were made every 24hr over 7 consecutive days (168h) and placed into a non-humidified benchtop incubator at 37°C (G185, K-systems). At the end of 7 days, all dishes were removed and osmolality of microdrops measured using a vapor pressure osmometer (Wescor). Positive controls included media directly out of the bottle (con) and microdrops under oil for 10min (con drops). The resulting 16 treatments were measured 3 times each. Data were analyzed using ANOVA and Tukey analysis and presented as the mean ±SEM, $p<0.05$.

RESULTS: Mean osmolality of con media and con drop were similar (263±1.0 and 265±2.1mOsm, respectively). Osmolality of microdrops under washed and unwashed oil increased over time and were both significantly higher than con and con drops after 48h of culture. No significant differences were apparent between washed and unwashed oil within any time point examined.

CONCLUSIONS: Washing of mineral oil for use with microdrop overlay had no protective impact on stabilizing media osmolality compared to unwashed oil when utilized for up to 7 days in a non-humidified incubator environment. Both types of oil overlay resulted in significant osmolality increase over time compared to controls after 48h of culture. This osmolality increase should be considered when trying to optimize growth conditions within the IVF laboratory.

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UNSTABLE OSMOTIC PRESSURE IN MICRODROPS CULTURED UNDER MINERAL OIL IN NON-HUMIDIFIED INCUBATORS. K. Iwata, K. Yumoto, Y. Mio. Reproductive Centre, Mio Fertility Clinic, Yonago, Japan.

OBJECTIVE: Humidified incubators are widely used for culturing human embryos, but in recent years, non-humidified benchtop incubators have been widely used due to their small size and lower risk of fungal contamination. Mineral oil is typically used to cover microdrops of culture medium in dishes so as to prevent changes in the osmotic pressure, pH, and temperature of the medium. The stability of pH and temperature in microdrops in non-humidified incubators has been verified, but it is unclear whether osmotic pressure remains stable. In this study, we cultured different size microdrops in humidified and non-humidified incubators to determine the stability of the osmotic pressure.

DESIGN: Basic Clinical study.

MATERIALS AND METHODS: We compared three incubators: (i) humidified benchtop, (ii) non-humidified benchtop, (iii) humidified water jacket. Microdrops (50μl, 100μl, 200μl) of a single step medium (A) [standard value (SV): 265±10 mOsm/kg] or a sequential medium (B) (SV: 290±10 mOsm/kg) were prepared in φ35 mm culture dishes (Nunc), and then covered with mineral oil (Naka Medical Co. Japan). After one and two days incubation, the osmotic pressure of the microdrops was measured using a micro-sample osmometer (Fiske 210®).

RESULTS: In the humidified benchtop and water jacket incubators, there were slight but non-significant increases of up to 4 mOsm/kg over 1 and 2 days in both media, regardless of drop volume. In contrast, there were significant increases in osmotic pressure in the non-humidified benchtop incubator. In medium A, the 50μl drops increased from 267 to 277±0.6 mOsm/kg after 1 day and to 283±0.9 mOsm/kg after 2 days, while in medium B, the 50μl drops increased from 289 to 306±0.7 mOsm/kg after 1 day and to 310±0.8 mOsm/kg after 2 days. The increase in osmotic pressure of the 100μl and 200μl drops was ~50% less than in the 50μl drops. In 100μl drops of medium A, osmotic pressure was 271±0.2 mOsm/kg after 1 day and 275±0.5 mOsm/kg after 2 days, while in medium B, it was 294±0.6 mOsm/kg after 1 day and 297±0.4 mOsm/kg after 2 day. In 200μl drops, the osmotic pressure was 271±0.7 mOsm/kg after 1 day and 274±0.4 mOsm/kg after 2 days, while in medium B, it was 293±0.6 mOsm/kg after 1 day and 299±0.5 mOsm/kg after 2 days. In the small drops (50μl), the increases in osmotic pressure in the non-humidified benchtop incubator were significantly greater than in the humidified incubators ($P<0.01$).

CONCLUSIONS: The osmotic pressure of microdrops incubated under a layer of mineral oil is stable in humidified incubators, but increased rapidly and significantly in the non-humidified benchtop incubator after only 1 or 2 days incubation. This suggests that a mineral oil overlay may not adequately protect small microdrops from evaporation in a non-humidified atmosphere. Furthermore, the significant increases in osmotic pressure observed in 50μl

Microdrop Osmolality (mOsm) After Time in Culture

	24h	48h	72h	96h	120h	144h	168h
Unwashed Oil	270.3±2.2	271.7±1.5	277.7±1.8	282.7±1.2	283.3±1.3	288.7±0.9	300.7±2.3
Washed Oil	270.7±1.3	271.3±0.3	278.3±2.1	284±1.2	284.3±0.9	291.7±0.9	299.7±0.9