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RESEARCH ARTICLE

Effect of Oxygen Concentration on Development and Quality of Embryos during Intracytoplasmic Sperm Injection (ICSI).

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Abstract

Background: Elevated and non-physiological O_2 concentrations could create unfavorable conditions in the substrate to produce free oxygen radicals that cause oxidative stress, which may be involved in the etiology of defective embryo development with higher rates of fragmentation.

Aim of the Work: to evaluate the effects of oxygen tension on the early development and quality of embryos during Intracytoplasmic sperm injection (ICSI) treatment cycles.

Patients and Methods: This prospective cohort observational study was carried out in the Embryology Laboratory of Assisted Reproduction Unit at Ain Shams University Maternity Hospital in the period from Octoberof2014 to October of 2017.This study comprised 1143 female gametes (oocytes) from 100 ICSI cycles patients. All the oocytes were injected by the same embryologist and were divided after injection equally into two groups according to system of incubation.

Results: Fertilization and division Rates were significantly lower among CO_2 incubator group than among Triplet incubator group, No significant difference between Implantation Rate of cases underwent incubation at atmospheric versus low oxygen tension and versus mix of both, No significant difference between outcomes in cases underwent incubation at atmospheric versus low oxygen tension and versus mix of both.

Conclusion: Our findings support the idea that human embryo culture in low oxygen tension will improve the fertilization and embryo development, and ART outcomes.

Introduction:-

The role of oxygen tension during the culture of gametes and embryos has been the subject of study in both animal models and humans (**Sobrinho DB et al.,2011)**[(1)],. Following protocols from somatic cell culture techniques, the embryos of humans and other mammals have traditionally been cultured under atmospheric oxygen tension (~20%). However, experimental studies in various species of mammals have revealed that the concentration of O2 inside the uterus and oviducts usually fluctuates in the range of 2-8%(**Wale PL, Gardner DK.,2015)**[(2)] .

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Culture at low levels of O2 (5-7%) can improve embryonic development in several species, including mice (**Rinaudo PF et al .,2006)**[3], rats, hamsters, rabbits, pigs, goats, sheep, and cattle**(Yuan YQ et al .,2003)**[4]. In addition, culturing at a low O2 concentration is associated with a reduced rate of aneuploidy in mouse embryos. In general, these results are associated with a reduction of the harmful effects of reactive oxygen species (ROS).

In oocytes and embryos, even with endogenous defense mechanisms (**Guerin P et al .,2001)**[5], disturbances in physiological processes can lead to an increase in the generation and accumulation of ROS, which are associated with various degrees of cell damage (DNA fragmentation, changes in gene expression and organelle and membrane disturbances)(**Kang JT et al .,2012)**[6]. Consequently, interrupted or delayed embryonic development, embryonic fragmentation, apoptosis or health impairment during pregnancy can be observed(**Kovacic B, Vlaisavljevic V.,2008)**[7]. ROS may originate either directly within gametes and embryos (by different enzymatic mechanisms) or from the environment in which they are located. In contrast, the in vitro manipulation of gametes in embryos favours the generation of ROS as it involves the exposure of eggs and embryos to xenobiotics, disturbed concentrations of metabolic substrates, traces of transitional elements, light and high oxygen concentrations (**Sobrinho DBetal .,2011)**[1].

On the other hand, the belief that the detrimental effects of atmospheric oxygen tension on embryonic development are caused from the increased production of ROS could be an inexact view of the role of oxygen during embryo development. The culture of early embryos at low O2 concentration can influence both cellular mechanisms and gene expression(**Edwards NA et al .,2016)**[8].

Assuming that in humans physiological hypoxia also exists in the female genital tract, the results from animal experiments have important implications for the clinical application of IVF/ intracytoplasmic sperm injection (ICSI) (**Kovačič B et al .,2010)**[9].

As reported in animal experiments, beneficial effects of reduced O2 levels have also been observed in human studies(**Yang CS et al .,2016)**[10], including a greater rate of embryonic development up to the blastocyst stage, a faster cleavage rate, an increased blastulation rate, an increase in the number of blastocyst cells and in the number of cryo-preserved blastocysts, and an increase in the proportion of high-quality blastocysts. Regarding clinical outcomes, some studies have reported improvements in the implantation rate, pregnancy rate, delivery and live births with low O2 concentrations compared with atmospheric concentrations(**Waldenstrom U et al .,2009)**[11].

Conversely, other randomized studies have failed to yield positive results, reporting no differences in the implantation and pregnancy rates. Therefore, while several trials have compared the effects of different O2 concentrations, definitive conclusions could not be drawn from the individual studies because of conflicting results. Whether the culture of human oocytes and embryos in low concentrations of oxygen can actually improve the clinical results of assisted reproduction cycles is a question that remains to be answered (**Kovacic B et al .,2010)**[12].

Subjects and Methods:-

prospective cohort observational study , conducted on100 patients (corresponding to 546 in group1 and 597in group 2day 3 embryo in each group) (undergoing ICSI at Assisted Reproduction unit - Ain Shams University Maternity Hospital Starting from October, 2015 to October, 2017.

Sample size justification

Depending on (Calzi F[1] et al.,2012)[13].who found that proportion of embryos obtained (38% vs. 50%, respectively) from low and normal tension , and assuming the power= 0.80 and α=0.05, and by using PASS 11th release the minimal sample size for an equal controlled is 268 oocytes in each group..

The aim of the study

The aim of the present study is to evaluate the effects of oxygen tension on the early development and quality of embryos during Intracytoplasmic Sperm Injection (ICSI) treatment cycles.

Settings:
Population:
100 patients (corresponding to **546** in group1 and 597in group2 day 3 embryos in each group) undergoing ICSI at Assisted Reproduction unit - Ain Shams University Maternity Hospital Starting from October, 2015 to October, 2017.

Subjects Recruitment
After the initial evaluation of the infertile couple upon presentation to the hospital, exclusion and inclusion criteria were applied. Couples, who met the inclusion criteria were invited to participate, adequately counseled and their informed consent obtained before their inclusion in the study.

Inclusion criteria included:
patients with ages ranging from 20 to 35 years old with retrieved oocytes more than 5 oocytes per cycle and BMI < 30 kg/m^2.

Exclusion criteria included:
Patients with congenital abnormalities in female oocytes and male factor with Teratozospermia were excluded from the study.

Research Methodology:
All patients were subjected to the following:-
1. Pre-enrollment assessment as per unit protocol
2. Controlled ovarian hyperstimulation and Intracytoplasmic Sperm Injection - embryo transfer cycle.

Treatment Plan
The standard protocol applied was long protocol to all patients under the study according to assisted reproduction unit policy. Patients started combined oral contraceptive Pills on first day of Menses then GnRH analogues (Decapeptyl 0.1mg-ferring pharmaceuticals, Germany) was started on day seventeen of cycle and continued till HMG was started on day two or three of next cycle for ovarian stimulation. Following Confirmation of Pituitary desensitization, defined as an endometrial thickness of <5mm and no ovarian activity evident on transvaginal ultrasound in association with an estradiol concentration <50pg/ml. First, follicullometry was done on day six of starting HMG and repeated every other day till the size of follicles reached 14mm then it was repeated daily till reaching the condition of having at least three follicles 18mm in size at which point the trigger with hCG was given at dose of 10,000IU (Choriomon 5000IU.IBSA.switherland) preparing for Ovum Pick up, trans vaginal ultrasound guided oocyte retrieval will be performed 34-36 hr. after hCG administration.

Oocytes collection
Retrieval of Oocytes was performed under transvaginal ultrasound guidance (Mindray, DP-8800 Plus, china). The aspirates were collected in pre-warmed tubes, and then oocytes were washed in pre warmed Global with HEPES buffered medium**.**

Denudation and assessment
The oocytes denudation was done under stereomicroscope (Zeiss stemi2000, Oberkoche, Germany with high magnification. and assessment of maturity was undertaken by inverted microscope(Nikon eclipse TE 2000-S,Tokyo,Japan). The nuclear status of denuded oocytes was subsequently recorded as Germinal vesicle (GV), Metaphase I (MI), Metaphase II (MII).

Oocyte Maturational Status
Maturational status of each oocyte at retrieval was determined by morphology assessment using inverted microscope, the overall appearance of the cytoplasm, zonapullicida and polar body, as well as the granulosa, cumulus, and corona companion somatic cells are considered.

Semen processing before ICSI
Semen sample were collected by masturbation after 2-7 days of abstinence. the preparation for ICSI was performed after complete liquefactions, the semen was washed with tissue culture media, and final pellet was re suspended in 0.2ml of sperm washing medium,(Irvine Scientific, Santa Ana,USA)until time of injection (WHO,2010).

Microinjection

Under Nikon Eclipse TE 2000-s occupied by Hoffman Modulation contrast, with the attached micromanipulator Integra -1(RI, Cornwell, Uk), microinjection took place. .All the oocytes were injected by the same embryologist and were divided after injection equally into two groups according to system incubation.

1. Group1: Gametes and embryos incubated throughout in(5%O2) Low Oxgyen tension from time of injection till day3 of culture.
2. Group 2:Gametes and embryos incubated throughout in (21%O2) atmospheric Oxgyen from time of injection till day3 of culture.
3. All incubators were performed at 37°c

Embryo culture and development

Injected oocytes were cultured in Global ® total medium with ovoil®overly in the falcon dishes, each inseminated oocyte incubated in one drop and put in incubators Low Oxgyen (5% O2) (labotect ® C200, Gottingen, Germany) adjusted to 37.0c, 6 %$CO^2$and 5%O_2& Co2 incubator (21% O2) to be visualized after 16-18hrs to check fertilization.

Assessment of Fertilization and cleavage stage embryo

Fertilization was assessed 18-20 hrs. from insemination /injection and confirmed by presence of 2 pronuclei (2PN) and the alignment of nuclear precursor bodies (NPB)**.**At the same time, the pronuclear morphological score was assessed**.**The observation of 2PN was performed using inverted microscope with Hoffman modulation contrast at X400 magnification (TE 2000 U, Nikon crop. USA).Zygotes were evaluated according to (Alpha Scientists in reproductive Medicine and ESHRE Special Interest Group of Embryology, 2011).

Day 5 Embryo Assessment (Blastocyst).

Similar to the cleavage stage embryos, embryo morphological features are very important in assessment of blastocyst quality and its reproductive potential. Assement was done according to blastocyst scoring system was proposed by Gardner (Gardner et al.,2000)**.**

Embryo transfer (ET)

The best quality embryo- maximum 2 embryos were transferred at day 5. Embryo transfer done guided by trans abdominal ultrasound in all cases.

Luteal phase support

Medication is prescribed for patient by oral, vaginal and intramuscular progesterone. After 9 day from embryo transfer serum Human chorionic gonadotrophins (HCG) measured, if positive test detected patient followed up with transvaginal ultrasound after 20 days for clinical pregnancy detection.

Results:-

Collected Oocytes 1218 yielded 1143MII, Randomized division to two groups: group (1)yield 546 oocyte in Co2 incubator (20%oxgyen) which yield (372)fertilized oocyte &(358) Division, group (2)yield 546 oocyte in Triplet incubator (5%oxgyen) which yield (597)fertilized oocyte &(424) Division, Then Randomized division for Embryo transfer yield (22) Casesfrom Co2 (20% O2) yield (8) cases Clinical Pregnancy,(36) Cases from Triplet(5%O2) yield (11)cases clinical pregnancy and(42)Casesfrom Both (Mixed Transfer) yield (15) cases Clinical Pregnancy.

Table (1):-Number of collected and incubated oocytes in the study population

	Finding
Total oocytes collected	1218
Total number of MII oocytes	1143
Total number of oocytes incubated in CO_2 incubator	546
Total number of oocytes incubated in triplet incubator	597
MII rate **MII*100/Collected**	93.8%

Number of collected, Metaphase II incubated oocytes in the study population. Represented as Mean ± SD, Range and Median (IQR) **Table (2):-**

Table (2): Number of collected and incubated oocytes in the study population

	Mean±SD	Range	Median (IQR)
Number of collected oocytes	12.2±3.2	6.0–22.0	12.0 (10.0–14.0)
Number of MII	11.4±3.0	6.0–22.0	11.0 (9.0–13.0)
Number of oocytes incubated in CO_2 incubator	5.5±1.5	3.0–11.0	5.0 (4.0–6.0)
Number of oocytes incubated in triplet incubator	6.0±1.5	3.0–11.0	6.0 (5.0–7.0)

Paired comparison of the oocytes incubated at atmospheric versus low oxygen tension
Number of oocytes undergoing fertilization, Number of oocytes undergoing normal fertilization, Total number of oocytes undergoing fertilization, Number of oocytes undergoing division, Number of grades III embryos produced and Total number of embryos were significantly lower among CO_2 incubator group than among Triplet incubator group. Number of grades I embryos produced was significantly higher among CO_2 incubator group than among Triplet incubator group.**Table (3):**

Table (3):-Paired comparison of the oocytes incubated at atmospheric versus low oxygen tension

Variables	CO_2 incubator		Triplet incubator		P
	Median	IQR	Median	IQR	
Number of oocytes undergoing fertilization	4.0	2.0–5.0	4.0	3.0–5.0	**0.004***
Number of oocytes undergoing normal fertilization	4.0	2.0–4.8	4.0	3.0–5.0	**0.006***
Number of oocytes undergoing abnormal fertilization	0.0	0.0–0.0	0.0	0.0–0.0	0.552
Total number of oocytes undergoing fertilization	4.0	2.0–5.0	4.0	3.0–5.0	**0.027***
Number of oocytes undergoing division	2.0	1.0–3.0	4.0	3.0–5.0	**0.004***
Number of grades I embryos produced	1.0	0.0–2.0	3.0	2.0–4.0	**<0.001***
Number of grades II embryos produced	0.0	0.0–1.0	0.0	0.0–1.0	0.886
Number of grades III embryos produced	4.0	2.0–5.0	4.0	3.0–5.0	**0.027***
Total number of embryos	4.0	2.0–4.8	4.0	3.0–5.0	**0.006***

Wilcoxon signed rank test, *Significant

Paired comparison of the oocytes incubated at atmospheric versus low oxygen tension, Fertilization and division Rates were significantly lower among CO_2 incubator group than among Triplet incubator group. **Table(4):**

Table (4):-Paired comparison of the oocytes incubated at atmospheric versus low oxygen tension

Variables	CO_2 incubator	Triplet incubator	P
Fertilization rate **Fertilized*100/injected**	68.1% (372/546)	71.1% (424/597)	**<0.001***
Division rate **Divided*100/fertilized**	96.2% (358/372)	98.3% (417/424)	**<0.001***

McNemar test, *Significant
Comparison between Implantation Rate of cases underwent incubation at atmosphericversus low oxygen tension and versus mix of both, No significant difference between Implantation Rate of cases underwent incubation at atmospheric versus low oxygen tension and versus mix of both **Table (5):**

Table (5):-Comparison between Implantation Rate of cases underwent incubation at atmospheric versus low oxygen tension and versus mix of both

	CO_2 incubator	Triplet incubator	Mixed	P
Implantation Rate **Embryos by US*100/Embryos transferred**	20.8% (10/48)	18.8% (16/85)	19.8 (20/101)	#0.960

#Chi square test
Comparison between outcomes in cases underwent incubation at atmospheric versus low oxygen tension and versus mix of both, No significant difference between outcomes in cases underwent incubation at atmospheric versus low oxygen tension and versus mix of both. **Table(6)**

Table (6):-Comparison between outcomes in cases underwent incubation at atmospheric versus low oxygen tension and versus mix of both

		CO_2 incubator	Triplet incubator	Mixed	P
Number of cases		**22**	**36**	**42**	
Day of transfer	**Day 3**	6 (27.3%)	13 (36.1%)	20 (47.6%)	#0.258
	Day 5	16 (72.7%)	23 (63.9%)	22 (52.4%)	
Total number transferred embryos		48	85	101	
Total number high grade transferred embryos		48	83	94	
Chemical pregnancy Rate		11 (50.0%)	13 (36.1%)	20 (47.6%)	#0.483
Clinical pregnancy Rate		8 (36.4%)	11 (30.6%)	15 (35.7%)	#0.861
Pregnancy number	**None**	14 (63.6%)	25 (69.4%)	27 (64.3%)	#0.258
	Single	6 (27.3%)	7 (19.4%)	10 (23.8%)	
	Twin	2 (9.1%)	3 (8.3%)	5 (11.9%)	
	Triplet	0 (0.0%)	1 (2.8%)	0 (0.0%)	

#Chi square test

Discussion:-

The delivery of healthy, normal-weight babies at term, resulting in healthy children and subsequently healthy adults, is the clear goal of assisted human conception.. The reasons for this may lie not only in overall improvements in IVF laboratories in recent years, but plausibly through the utilization of 5% oxygen for extended culture; those countries and clinics utilizing physiological concentrations of oxygen reporting no adverse effects on the children born following extended culture(**Chambers GM et al .,2015)**[14]. Interestingly, in the meta-analysis considered by Maheshwari and colleagues, those studies reporting negative outcomes with extended culture appear to be from clinics/countries in which 20% oxygen is typically employed (**Christianson et al .,2014)**[15].

The toxic effects of oxygen on the embryos of various animal species are reviewed. Methodologies for assessing embryonic damage are discussed and possible ways of preventing the damage are explored. Three methods of potentially minimizing oxidative damage to human embryos were tested using gametes, zygotes, and embryos from a clinical IVF programme: (i) decreasing the oxygen tension in the gas phase used for culture during insemination, fertilization, and embryo growth; (ii) changing the formulation of culture media to include some components designed to protect against oxidative damage; and (iii) reducing the duration of insemination to minimize the effect of oxidative damage caused by spermatozoal metabolism. Fertilization, cleavage, embryo utilization, pregnancy, and embryo implantation rates were used to monitor these changes. Although all three methods gave an increase in success rates, there was still a dramatic decrease in success with patient age. It is suggested that, although the system of handling and culturing embryos can be optimized with respect to embryonic mitochondrial function, there are inherent age-related defects in oocytes and embryos that are still more fundamental than the environmental conditions of the embryo(**Catt J and Henman M .,2000)**[16].

The present study focused on the Effect of Oxygen Concentration on Development and Quality of Embryos during Intracytoplasmic Sperm Injection (ICSI). This study comprised 1143 female gametes (oocytes) from 100 ICSI cycles patients. All the oocytes were injected by the same embryologist and were divided after injection equally into two groups according to system incubation.

1. Group1: Gametes and embryos incubated throughout in (5%O2) tension from time of injection till day3 of culture.
2. Group 2:Gametes and embryos incubated throughout in (21%O2) atmosphere from time of injection till day3 of culture.

All incubators were performed at 37°c

IVF laboratories performing these procedures must comply with strict requirements to control the conditions to which embryo cultures are exposed, including temperature variations, light, and O_2 and CO_2 pressure (**Cohen J,et al .,1997)**[17]. It is also important to control for substances that might affect the development of the embryos, such as dust particles, VOC, and disinfectants.

(Peng ZF et al.,2015)[18]found that Embryos from the 5% O2 group yielded higher rates of fertilization and implantation compared to those from the 20% of O2 group, Embryos resulting from the 20% O2 tension group

yielded lower cleavage rates as compared to those from the 5% of O2 group which agreed with our study Fertilization and division Rates were significantly lower among CO_2 incubator(20%o2) group than among Triplet incubator group(5% o2).

(Guo et al., 2014)[19]have found no significant differences were found between the two groups with respect to their fresh cycle characteristics such as clinical pregnancy rate (CPR) and implantation rate (IR). This agreed with our study as there was No significant difference between Implantation Rate or, pregnancy rate (CPR) and of cases underwent incubation at atmospheric versus low oxygen tension and versus mix of both.

In contrast (**Kovacic B et al.,2010)[12]**reported that culturing embryos in low oxygen concentration improves embryo utilization rate and increases the chance of pregnancy. Kovacic et al. **[12]**reported that low oxygen is recommended for poor responder patients with more optimal embryos to select (62.5%. in 5 %O2 vs. 44.7% in 20% O2).

In current study we observed that the Number of grades I embryos produced was significantly higher among triple gas (5% oxygen)while significantly lower among CO_2 incubator(20% oxygen) group than among Triplet incubator group(5% oxygen). This agreed with**(Guo et al., 2014)[18]** as they found that embryos of low oxygen group on day 3 had higher percentage of 8 blastomeres and lower percentage of less than 5 blastomeres compared to atmospheric oxygen group.

(Sobrinho DB et al.,2011)[1]found that fertilization rates did not differ significantly between the group of oocytes that was cultured at OC~5.in contrast to our study Fertilization and division Rates were significantly lower among CO_2 incubator group than among Triplet incubator group. In other hand the general implantation rates did not differ between the group of patients that received sets with only OC~5 embryos and the group receiving only OC~20 embryos and this agreed with our study.

(**Wale PL and Gardner DK et al .,2010)[20]**clearly showed that the zygote stage is most sensitive to oxygen toxicity. Exposure of embryos to atmospheric oxygen results in irreversible damage, such as a delay in cleavage divisions.

(Bedaiwy MA et al .,2004)[21]reported that high ROS levels on day 1 in the culture media are associated with a low fertilization rate, low cleavage rate, and high embryonic fragmentation during ICSI cycles].

VitROShield is the combination of hyaluronan, gentamicin and the antioxidant lipoate in the culture system and protects from pH induced stress, free oxygen radicals, infections and cryo damage. Antioxidant activity neutralizes the ROS and negates the effect on fertilization and cleavage rates in the first two cleavage divisions **(Guo et al., 2014)[19]**.

Several other lines of evidence would suggest that low O2 concentrations are more suitable culture conditions for pre implantation embryos. Embryos develop in vivo under low O2 levels, as in the oviduct and uterus of various mammalian species O2 concentrations have been reported to be ~11–60 mmHg, which corresponds to ~1.5–9% O2

((Fischer and Bavister, 1993)[22]. Furthermore, a reduced O2 concentration of 5% has been shown to result in a slightly decreased formation of reactive oxygen species in mouse embryos as compared to 20% O2. Reactive oxygen species have been implicated in the retardation of early embryo development in vitro (**Trokoudes KM, et al .,2005)[23]**.

The majority of studies thus indicate that culture under low O2 is beneficial for complete pre implantation embryonic development in vitro. However, little is known about the effect of a lower O2 concentration during the first 2 or 3 days of in vitro development on post implantation embryonic development and pregnancy rates(**(Na Guo et al., 2014)[19]**.

The present study shows that, although culture under 5% O2 indeed leads to slightly improved pre implantation embryonic viability in the human, this effect is either too marginal to result in higher pregnancy rates, or low O2 concentrations exert an effect during the later stages of pre implantation development only. To conclude, in a culture system of microdroplets of medium under oil, no beneficial effect on fertilization, embryonic development and pregnancy rates of culturing human oocytes and embryos for 2 or 3 days under 5% O2 as compared to atmospheric

O2 concentrations was found. Only when embryos were cultured in vitro during the total pre implantation period, was a slight but significant improvement of development to the blastocyst stage found when 5% O2 was used.

Given the mild differences in outcome observed between embryos incubated in low or high O2 tension conditions it may be premature to recommend the use of 5% O2 for oocyte and/or embryo culture. Moreover, given that to achieve a low oxygen concentration, an incubator requires both CO2 and O2 sensors and the ability to supply three gases (N2, CO2 and O2) simultaneously, this increases the risk of fluctuations during embryo culture. For instance, opening the incubator door to handle oocytes/ embryos requires re-equilibration of the inside atmosphere with a balanced mixture of gases to maintain a low O2 concentration, which may take longer than when only CO2 is used (**Trokoudes KM et al .,2005)**[23].

Nonetheless, there may be instances in which the use of low O2 tension could be highly advantageous and possibly overcome these drawbacks. In this regard, one study suggested that patients with poor prognosis (e.g. repeated implantation failures and poor responders with a low number of oocytes), as well as patients with advanced age (> 40 years old), benefited from transferring day 3 embryos cultured in a low O2 tension atmosphere(**Bavister B.,2004)**[24]. Therefore, the benefits and disadvantages of both systems may be outweighed in a case-to-case basis potentially driven by patient demographics in a particular clinical setting.

Conclusion:-

Our findings support the idea that human embryo culture in low oxygen tension will improve the fertilization and embryo development, and ART outcomes.

Recommendations:

The result demonstrated from this study illustrate that when embryos were cultured in vitro during the total pre implantation period, was a slight but significant improvement of development to the blastocyst stage found when 5% O2 was used.

So, we recommended that using time-lapse monitoring, to evaluate the temporal effect of different oxygen concentrations on the exact timing of human embryonic development & fluctuation occurs during manipulating of embryos.

References:-

1. **Sobrinho DB, Oliveira JB, Petersen CG, Mauri AL, Silva LF, Massaro FC, Baruffi RL, Cavagna M, Franco JG.** IVF/ICSI outcomes after culture of human embryos at low oxygen tension: a meta-analysis. Reproductive Biology and Endocrinology. 2011;9(1):143.
2. **Wale PL, Gardner DK.**The effects of chemical and physical factors on mammalian embryo culture and their importance for the practice of assisted human reproduction.Human reproduction update. 2015;22(1):2-2.
3. **Rinaudo PF, Giritharan G, Talbi S, Dobson AT, Schultz RM.** Effects of oxygen tension on gene expression in preimplantation mouse embryos.FertilSteril 2006, 86:1252-1265, 1265 e1251-1236.
4. **Yuan YQ, Van Soom A, Coopman FO, Mintiens K, Boerjan ML, Van Zeveren A, de Kruif A, Peelman LJ:** Influence of oxygen tension on apoptosis and hatching in bovine embryos cultured in vitro. Theriogenology 2003, 59:1585-1596.
5. **Guerin P, El Mouatassim S, Menezo Y.** Oxidative stress and protection against reactive oxygen species in the pre-implantation embryo and its surroundings. Hum Reprod update 2001, 7:175-189.
6. **Kang JT, Atikuzzaman M, Kwon DK, Park SJ, Kim SJ, Moon JH, Koo OJ, Jang G, Lee BC.**Developmental competence of porcine oocytes after in vitro maturation and in vitro culture under different oxygen concentrations.Zygote. 2012;20(1):1-8.
7. **Kovacic B, Vlaisavljevic V:** Influence of atmospheric versus reduced oxygen concentration on development of human blastocysts in vitro: a prospective study on sibling oocytes. Reprod Biomed Online 2008, 17:229-236.
8. **Edwards NA, Watson AJ, Betts DH.** P66Shc, a key regulator of metabolism and mitochondrial ROS production, is dysregulated by mouse embryo culture. MHR: Basic science of reproductive medicine. 2016;22(9):634-47.
9. **Kovačič B, Sajko MČ, Vlaisavljević V.**A prospective, randomized trial on the effect of atmospheric versus reduced oxygen concentration on the outcome of intracytoplasmic sperm injection cycles.Fertility and sterility. 2010;94(2):511-9.

10. **Yang CS, Zhang J, Zhang L, Huang J, Wang Y.** Mechanisms of body weight reduction and metabolic syndrome alleviation by tea. Molecular nutrition & food research. 2016;60(1):160-74.
11. **Waldenstrom U, Engstrom AB, Hellberg D, Nilsson S.** Low-oxygen compared with high-oxygen atmosphere in blastocyst culture, a prospective randomized study. FertilSteril 2009, 91:2461-2465.
12. **Kovacic B, Sajko MC, Vlaisavljevic V.** A prospective, randomized trial on the effect of atmospheric versus reduced oxygen concentration on the outcome of intracytoplasmic sperm injection cycles.FertilSteril 2010, 94:511-519.
13. 13.CALZI F[1], PAPALEO E, RABELLOTTI E, OTTOLINA J, VAILATI S, VIGANÒ P, CANDIANI M.EXPOSURE OF EMBRYOSTO OXYGEN AT LOW CONCENTRATION IN A CLEAVAGE STAGE TRANSFER PROGRAM: REPRODUCTIVE OUTCOMES IN A TIME-SERIES ANALYSIS;2012
14. **Chambers GM, Chughtai AA, Farquhar CM, Wang YA,** 2015. Risk of preterm birth after blastocyst embryo transfer: a large population study using contemporary registry data from Australia and New Zealand. Fertil.Steril.104, 997–1003.
15. **Christianson, M.S., Zhao, Y., Shoham, G., Granot, I., Safran, A., Khafagy, A., Leong, M., Shoham, Z.,** 2014. Embryo catheter loading and embryo culture techniques: results of a worldwide Webbased survey. J. Assist. Reprod. Genet. 31, 1029–1036.
16. **Catt J and Henman M.** Human Reproduction, 2000; Vol. 15, (Suppl. 2), pp. 199-206.
17. **Cohen J, Gilligan A, Esposito W, Schimmel T, Dale B.** Ambient air and its potential effects on conception in vitro. Hum Reprod 1997;12:1742–9.
18. **Peng ZF, Shi SL, Jin HX, Yao GD, Wang EY, Yang HY, Song WY, Sun YP.** Impact of oxygen concentrations on fertilization, cleavage, implantation, and pregnancy rates of in vitro generated human embryos. International journal of clinical and experimental medicine. 2015;8(4):6179.
19. **Guo, N., Li, Yu., Ai, J., Gu, L., Chen, W., and Liu, Q.,** *Int.J. Clin. Exp. Pathol*., 2014, vol. 7, no. 9, pp. 6191–6198.
20. **Wale PL and Gardner DK.** Time-lapse analysis of mouse embryo development in oxygen gradients.Reprod Biomed Online 2010; 21: 402-410.
21. **Bedaiwy MA, Falcone T, Mohamed MS, Aleem AA, Sharma RK, Worley SE, Thornton J and Agarwal A.** Differential growth of human embryos in vitro: role of reactive oxygen species. FertilSteril2004;82, 593-600.
22. **Fischer B, Bavister BD** 1993 Oxygen tension in the oviduct and uterus of rhesus monkeys, hamsters and rabbits. *Journal of Reproductionand Fertility* **99**, 673–679.
23. **Trokoudes KM, Minbattiwalla MB, Kalogirou L.** Controlled natural cycle IVF with antagonist use and blastocyst transfer. Reprod Biomed Online 2005; 11: 685-689.
24. **Bavister B.** Oxygen concentration and preimplantation development. Reprod Biomed Online 2004; 9: 484-486.