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Maternal and Perinatal Morbidity in Second Stage Versus First Stage Cesarean Sections in Sultan Qaboos University Hospital

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Abstract- Introduction: Over the last few decades, cesarean sections have been increased dramatically due to several medical and non-medical reasons. We can classify cesarean sections in emergency into either of the two stages of labor; at the first stage in which the cervix is dilated but not fully or at the second stage where the cervix is fully dilated. Studies revealed that the second stage cesarean sections are associated with a higher risk of maternal morbidities such as, intraoperative trauma and hemorrhage, which increase the need for blood transfusion. Also, some fetal morbidities can manifest, such as low APGAR score and umbilical artery pH at birth. This study aimed to assess the maternal and perinatal morbidity in the second stage cesarean sections compared to the first stage cesarean sections in Sultan Qaboos University Hospital.

Materials and Methods: This is a retrospective cross-sectional study. The study included all emergency cesarean sections on both stages of labor done in SQUH during a three years from January 2015 to December 2017. Maternal and neonatal characteristics and outcomes were obtained from delivery ward registers and Hospital Information System, which were analyzed later. Bar charts were used to display the prevalence. The continuous variables were tested by t- test and Mann-Whitney U test.

Keywords: cesarean sections, second stage, maternal, neonatal, morbidities.

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Maternal and Perinatal Morbidity in Second Stage Versus First Stage Cesarean Sections in Sultan Qaboos University Hospital

Mallak Alkalbani ^α & Mariam Mathew ^ο

Abstract- Introduction: Over the last few decades, cesarean sections have been increased dramatically due to several medical and non-medical reasons. We can classify cesarean sections in emergency into either of the two stages of labor; at the first stage in which the cervix is dilated but not fully or at the second stage where the cervix is fully dilated. Studies revealed that the second stage cesarean sections are associated with a higher risk of maternal morbidities such as, intraoperative trauma and hemorrhage, which increase the need for blood transfusion. Also, some fetal morbidities can manifest, such as low APGAR score and umbilical artery pH at birth. This study aimed to assess the maternal and perinatal morbidity in the second stage cesarean sections compared to the first stage cesarean sections in Sultan Qaboos University Hospital.

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Results: Out of 172 cesareans sections, 93 (54.3%) were done during the first stage of labor, and 79 (45.9%) were during the second stage of labor. Second stage cesarean sections are associated with higher rate of maternal and neonatal morbidities compared to first stage cesareans. The rate of intraoperative hemorrhage (9.0% vs. 1.1%), the extension of the uterine incision (10.1% vs. 1.1%) and the need for blood transfusion (73.4% vs. 37.6%) are significantly higher in second stage cesareans. The mean length of hospital stay is significantly higher in the second stage cesareans. The babies born by second stage cesareans have a lower mean umbilical artery pH (7.22).

Conclusion: Intraoperative hemorrhage, the extension of uterine incision, increased need for blood transfusion and low neonatal arterial cord pH were the most morbidities associated with second-stage cesarean sections in SQUH. Further prospective multicentric studies with more sample size should be done.

Keywords: cesarean sections, second stage, maternal, neonatal, morbidities.

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I. INTRODUCTION

a) Cesarean sections

Over the last few decades, cesarean sections have been increased dramatically due to several medical and non-medical reasons (1). The average rate of the cesarean sections in Oman has raised progressively from 9.7% in 2000 to 15.7% in 2009 (2). In Sultan Qaboos university hospital, the cesarean sections rate is similar to those at developed countries (2). Therefore, considerable attention has been devoted to cesarean sections as it has more adverse outcomes on the mother and baby than vaginal delivery (3), (4), (5). The worldwide increase in the cesarean section rate has been attributed to some risk factors such as; high maternal Body mass index (BMI), advanced maternal age, high birth weight, previous cesarean sections, pre-pregnancy diabetes, mal presentations and abnormal positions of the fetus. Despite these factors may be predictable, they cannot be changed in labor (3), (6), (7).

b) Types of cesarean sections

Cesarean delivery comprises two types that are elective operations and emergency operations. Elective cesarean sections are planned cesareans, whereas emergency cesarean sections are performed due to obstetric emergencies. The types of cesarean sections are linked with different degrees of morbidities. In contrast to elective cesarean section, the emergency cesarean section is riskier on mother (8). Emergency cesarean sections which are done in labor could be at either of the two stages of labour; in the first stage in which the cervix is dilated but not fully or in the second stage where the cervix is completely dilated. Fetal distress, failure to progress, and dystocia are main causes for emergency cesarean sections at both stages of labor (9), (10).

c) Emergency cesarean sections comorbidities

Many existing studies revealed that the second stage cesarean sections are associated with a higher risk of maternal and perinatal morbidities than the first stage cesareans (3), (10), (11) and (12). Vousden et al. (2014) reported that second stage cesarean sections have a higher probability of intraoperative trauma such as a laceration to bladder or bowel or extension of the

uterine incision. Furthermore, women who underwent second stage cesarean sections have a higher risk of hemorrhage, which increases the need of blood transfusion (8), (10), (11).

Alongside maternal morbidities, emergency cesarean sections are associated with a higher risk of adverse outcomes on babies. In contrast to the first stage, there is a consensus that the babies who born by the second stage cesarean sections are more likely to be admitted to the Special Care Baby Unit (SCBU), because they probably have low APGAR score and umbilical artery pH at birth (10), (11).

Murphy et al. (2001: pp.1207) reveal that "women were less likely to proceed to the cesarean section or to have a major hemorrhage if they were managed by a senior operator." Therefore, skills and knowledge are required to reduce the number of emergency cesarean sections and adverse

There were no studies conducted in Oman to compare maternal and perinatal morbidity between second versus first stage cesarean sections. Thus, the aim of this study was to assess the maternal and perinatal morbidity in the second stage cesarean sections compared to the first stage cesarean sections in Sultan Qaboos University Hospital (SQUH), which advanced our understanding on this topic and will serve as a platform for future studies in this field.

II. MATERIALS AND METHODS

a) Study design and sample

This study was a retrospective cross-sectional study which included pregnant women who had emergency cesarean sections in the first stage of labor and second stage of labor at Sultan Qaboos University Hospital (SQUH), during a period of three years from January 2015 to December 2017.

Medical Record Numbers of women who underwent emergency cesarean sections in the first and second stage of labor during the study period were obtained from the delivery ward registers, and the required data was gathered from the Obstetrics and Gynecology department and Neonatal Unit through Hospital Information System (Track Care system). The data was saved in a secured excel sheet. Ethical approval from Sultan Qaboos university Ethics and Research committee was obtained before the data collection.

Exclusion criteria: Women with multiple cesarean sections, multiple pregnancies, fetal anomalies, intrauterine growth restriction, premature labor and fetal malpresentation were excluded from this study.

The collected data included maternal and neonatal characteristics such as maternal age, body mass index, gestational age, parity and dilatation of cervix at cesarean, type of anesthesia used, neonatal birth weight, and gender. Data on maternal morbidity included intensive care unit admission, blood loss, need

for blood transfusion, Intra-operative complications, wound infection, operative duration, and the length of hospital stay. Neonatal morbidity included APGAR score at 1 and 5 min, arterial cord pH, birth asphyxia, neonatal trauma, neonatal sepsis, and neonatal intensive care admission.

b) Statistical analysis

The data was analyzed using Statistical Package for the Social sciences (SPSS) version 23.0. Descriptive statistics were obtained and displayed in tables to represent the continuous variables. Bar charts were used to display the percentage of maternal and neonatal morbidities.

One sample Kolmogorov-Smirnov test was used to test the normality of the continuous variables. To test the difference in the variables between the two stages of cesarean sections, t-test was used for normally distributed continuous variables (arterial cord pH, APGAR score at 1 and 5 minutes) and Mann-Whitney U test for the continuous variables that do not follow the normal distribution. Chi-square test was used for categorized variables. Significance was considered when p-values were ≤ 0.05 . The analysis was done under the supervision of statisticians.

III. RESULTS

The sample size of this study included 172 women who underwent cesarean section during the period between January 2015 and December 2017. About 93 (54.1%) of the cesarean sections were done during the first stage of labor and 79 (45.9%) were during the second stage of labor.

a) Maternal and neonatal characteristics

Table 1: Maternal characteristics by stage of labor

Parameters	First stage cesarean sections (total number = 93)	Second stage cesarean sections (total number = 79)
	Mean \pm Standard Deviation (SD)	Mean \pm Standard Deviation (SD)
Age (years)	28 \pm 4	28 \pm 5
BMI (Kilogram/ meter ²)	33.7 \pm 6.6	31.5 \pm 6.4
Gestational age (weeks)	39 \pm 1	39 \pm 1
Cervical dilation on decision to operate (cm)	5.6 \pm 2.3	10.0 \pm 0
Parity:	Number (%)	
Primigravida	74 (79.6%)	48 (60.8%)
Multigravida	19 (20.4%)	31 (39.2%)

The demographical data of the mothers in both stages is presented in Table 1. The mean age of women delivered by first stage cesarean section is 28 years, which is similar to the mean age of second stage cesarean patients. Patients who underwent cesarean sections in the first stage of labor have average body mass index of 33.7 Kg/m², which is higher than the

second stage cesarean patients. However, the difference is not significant with P-value less than 0.05. Gestational age of women in both stages is 39 weeks. The mean cervical dilation on the decision to operate in first stage cesarean sections is 5.6 cm. The prevalence of primigravida is 79.6% and 60.8% in the first and second stages of labor, respectively.

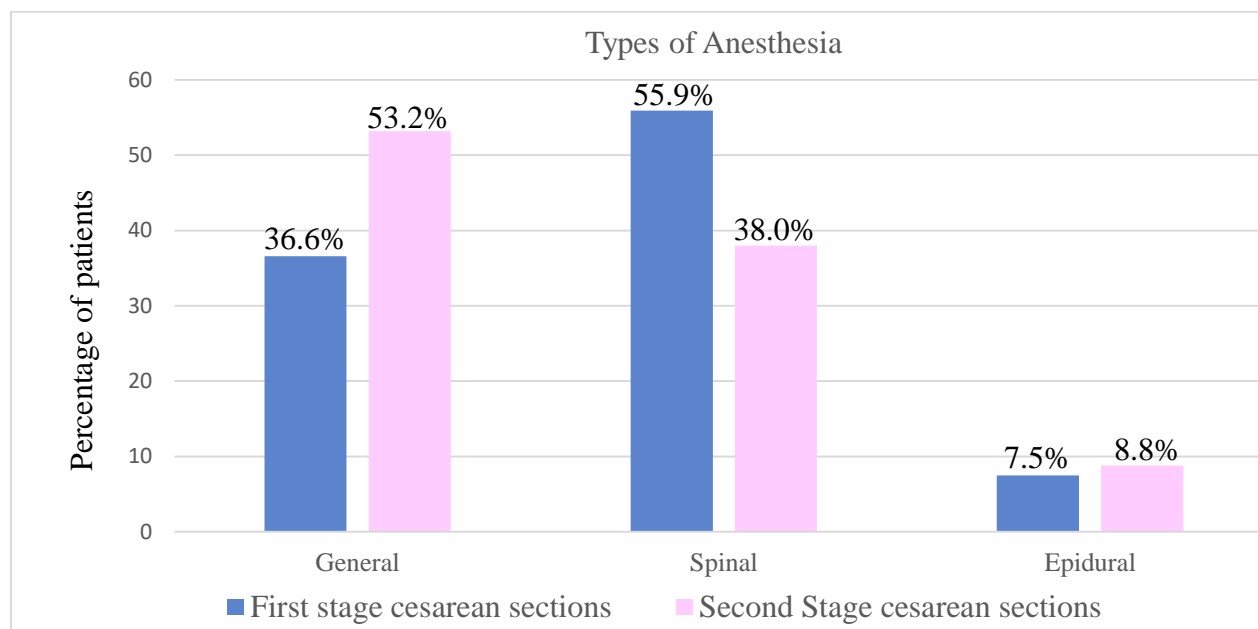


Figure 1: Type of Anesthesia used for Cesarean sections

In the first stage cesareans, the majority of mothers received spinal anesthesia 52 (55.9%). Approximately, 34 (36.6%) of them had general anesthesia. The rest of the first stage cesarean sections 7 (7.5%) were done under epidural anesthesia.

In the second stage cesarean sections, general anesthesia was received by 42 (53.2%) women. Also, 30 (38%) of second stage cesarean sections were done under spinal anesthesia, and 7 (8.8%) were done under epidural anesthesia as shown in Figure 1.

Table 2: Neonatal characteristics and stage of cesarean section

Parameters	First stage cesarean sections (total number = 93)		Second stage cesarean sections (total number = 79)	
	Male	Female	Male	Female
Baby Gender	Number (%)			
	51 (54.8)	42 (45.2%)	48 (60.8%)	31 (39.2%)
Baby Birth Weight (Kilogram)	Mean \pm Standard Deviation			
	3.3 \pm 0.5		3.3 \pm 0.3	

In the first stage cesarean sections, 51 (54.8%) babies were males and 42 (45.2%) were females. In the second stage cesarean sections, 48 (60.8%) babies

were males and 31 (39.2%) were females. The average weight of babies in both groups was similar to 3.3 Kg, as shown in Table 2.

b) Maternal outcomes

Table 3: Maternal Outcomes in the first stage and second stage cesarean sections

	First stage cesarean sections (Total number = 93)			Second stage cesarean sections (Total number = 79)			p- value
	Mean \pm SD	Minimum value	Maximum value	Mean \pm SD	Minimum value	Maximum value	
Blood loss (milliliter)	582.4 \pm 230.1	200.0	1800.0	656.8 \pm 334.7	250.0	2000.0	0.354
Pre-surgery Hemoglobin (gram/deciliter)	11.6 \pm 1.4	8.0	15.3	11.1 \pm 1.4	8.0	15.0	0.022
Post-surgery Hemoglobin (gram/deciliter)	10.1 \pm 1.3	6.8	13.3	9.7 \pm 1.3	7.4	13.5	0.043
Operation duration (Minutes)	59.3 \pm 16.9	36	103	59.5 \pm 19.2	36	120	0.732
Hospital stay (days)	2.99 \pm 0.68	2	7	3.32 \pm 0.89	2	7	0.05

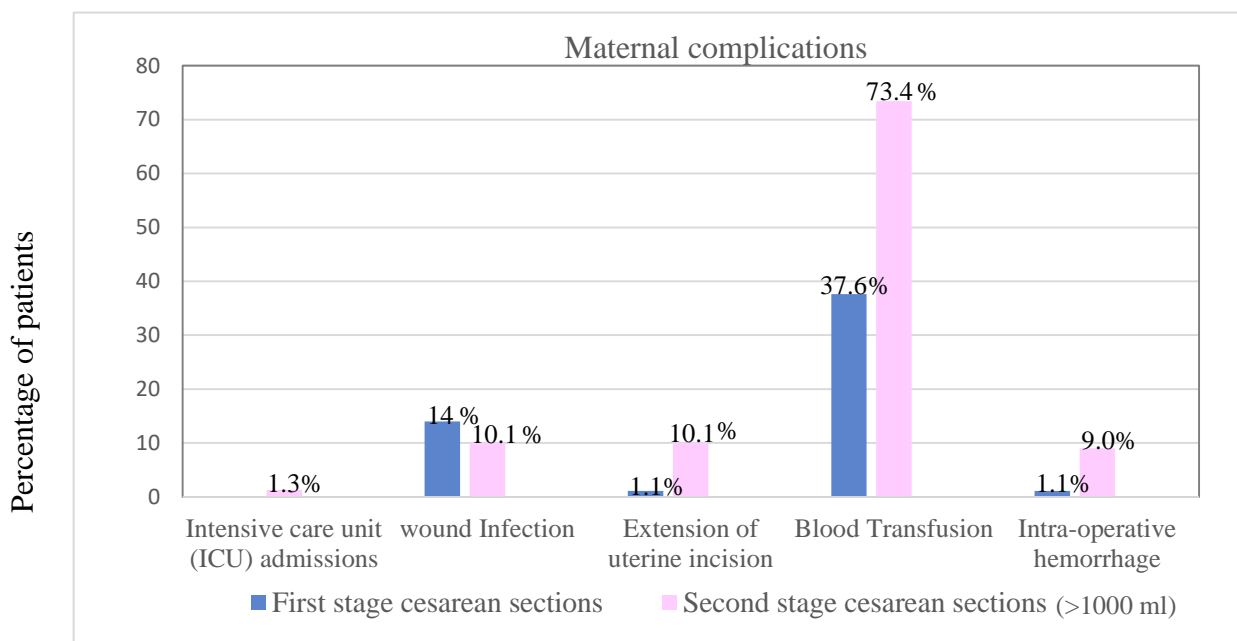


Figure 2: Maternal post-operative complications by stage of cesarean section

Table 3 represents maternal outcomes in the two types of cesarean sections, and Figure 2 represents maternal postoperative complications. The mean blood loss in women who underwent second stage cesarean sections is found to be higher (656.8 \pm 334.6 ml) than the mean of the first stage (582.4 \pm 230.1 ml). However, the difference is not significant as the p-value is 0.354 (more than 0.05). Pre-surgery mean hemoglobin is significantly lower in second stage cesarean sections (11.1 g/dl) and the P-value was 0.022. Post-operative mean hemoglobin is also significantly lower in the second stage cesarean sections (9.74 g/dl) with a p-value of 0.043. Women who underwent second stage cesarean sections have a significantly higher frequency of intraoperative hemorrhage (7 (9.0%)), with a p-value

of 0.024. Thus, 58 (73.4%) women who underwent a second stage cesarean section needed a blood transfusion. In contrast to the second stage cesareans, there are less blood transfusion needed 35 (37.6%) for first stage cesarean sections women. The difference is significant (p-value < 0.001).

The average time needed to perform the cesarean section is almost equal in both stages, 59.3 minutes in the first stage, and 59.5 minutes in the second stage. The second stage cesareans required a significantly longer hospital stay a mean of 3.32 days, with a p-value of 0.05.

One woman (1.3%) from second stage cesareans was admitted to the Intensive care unit (ICU) and none from the first stage, which is not significant

(p-value = 0.46). Thirteen patients (14%) of the first stage cesarean sections and eight patients (10.1%) of second stage cesarean sections had wound infection after the operation; the difference is not significant (p-value = 0.19). The number of extension of the uterine

incision is significantly higher in second stage cesareans (8 (10.1%)), (p-value = 0.12). There were no cases of thromboembolism and visceral injury in both groups.

c) Neonatal outcomes

Table 4: Neonatal outcomes in the first stage and second stage cesareans

Parameters	First stage cesarean sections (total number = 93)			Second stage cesarean sections (total number = 79)			p - value
	Mean \pm SD	Minimum value	Maximum value	Mean \pm SD	Minimum value	Maximum value	
Umbilical artery pH	7.28 \pm 0.11	6.9	7.9	7.22 \pm 0.10	6.9	7.6	0.020
APGAR score at 1 minute	8.28 \pm 1.35	4	9	8.03 \pm 1.76	3	9	0.297
APGAR score at 5 minutes	9.58 \pm 0.74	7	10	9.61 \pm 0.89	4	10	0.830

Table 4 shows that babies delivered by second stage cesareans have a significantly lower mean arterial cord pH (7.22) compared to first stage cesareans (7.28),

with a p-value of 0.02. There is no significant difference between stages in mean APGAR scores at both 1 and 5 minutes (p- values > 0.05).

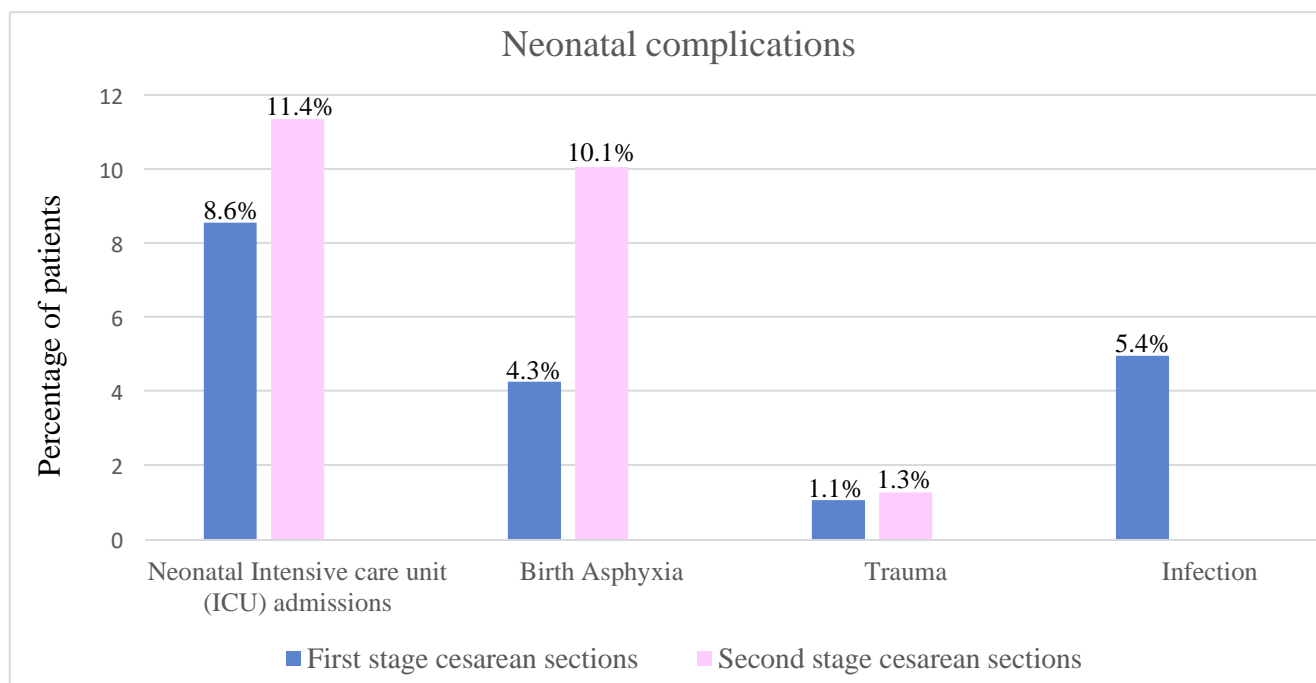


Figure 3: Percentage of Neonatal complications by stage of cesarean section

In figure 3, it is shown that neonates who were born by second-stage cesarean section have higher rates of Neonatal Intensive Care Unit (NICU) admissions 9 (11.4%) and Birth asphyxia 8 (10.1%). There is only one baby in each stage that has trauma. The difference between stages is not significant in all neonatal complications (p-value > 0.05).

IV. DISCUSSION

Over the last few decades, cesarean sections have been increased dramatically due to various reasons. Cesarean delivery can be either elective or emergency operations. Emergency cesarean sections could be either at the first stage or the second stage of

labor. Literature reveals more morbidities associated with second-stage cesarean sections compared to the first stage cesareans as shown in our study.

Unlike Asicioglu *et al.*, our study revealed that the difference between first and second stage cesarean sections in mean blood loss was not significant. While, Asicioglu *et al.* revealed a significant increase in mean blood loss in second stage cesarean sections. Despite that blood loss difference is not significant in our study; it is found that intraoperative hemorrhage is significantly higher in second stage cesarean sections. Thus, the need for blood transfusion is also higher in second stage cesarean sections. These findings are supported by existing studies that revealed the same findings

(Bashir *et al.*, 2018), (Asicioglu *et al.*, 2014), (Vousden *et al.*, 2014).

Moreover, our results reveal that the extension of uterine incision cases were considerably higher in the second stage cesarean sections. This result supports the existing evidence of an increase in extension of uterine incision in the second stage cesarean sections (15), (11), (14), (13).

Based on this study, the prolonged hospital stay needed in second stage cesarean sections due to more complications is in line with the findings of Asicioglu *et al.* study. On the other hand, Lurie *et al.* reported no significant difference in length of hospitalization between the two stages of emergency cesarean patients. Thus, more studies are needed to test the difference in-hospital stay in the second versus the first stage cesarean sections.

Second-stage cesarean section babies had lower arterial cord pH in our study and other studies as well (10), (11). These studies also recorded lower APGAR score, more birth asphyxia and trauma thus more Neonatal Intensive Care Unit admissions among second stage cesarean section babies, unlike our results that reported no significant difference.

Asicioglu *et al.*, (2014) reported that "A cesarean delivery performed during the second stage of labour is technically difficult because the fetal head engagement in the maternal pelvis has already been completed, and the maternal uterine muscle is very thin and tense. Additionally, the identification of the bladder and the low segment of the uterus is very difficult and birthing relatively larger infants is more difficult and traumatic", which explains the findings. The non-significant findings in our study is probably due to the small sample size.

V. CONCLUSION

In conclusion, Intraoperative hemorrhage, the extension of uterine incision, increased need for blood transfusion, and low neonatal arterial cord pH were the most common morbidities associated with second-stage cesarean sections in SQUH. Other findings were not significant.

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Severe Oligohydramnios with Good Fetal Outcome- A Case Report

By Dr. Sampada & Dr. Tushar Palve

Introduction- Amniotic fluid provides a protected milieu for the growing fetus, cushioning the fetus against mechanical and biological injury, supplying nutrients and facilitating growth and movement.

Oligohydramnios is defined as a single pocket measuring 2 cm in both vertical and horizontal planes or AFI less than 5 cm.

Incidence of oligohydramnios varies between 0.5 to > 5 %.

Causes of oligohydramnios:

- Maternal – postdated pregnancy, PPRM, hypertension, autoimmune disorders and maternal medications like prostaglandin synthetase inhibitors.
- Fetal- IUGR, fetal anomalies particularly of renal tract. Most commonly associated include bilateral renal agenesis, multicystic dysplastic kidneys, bladder outlet obstruction and infantile polycystic kidney disease.
- Other factors noted to affect are maternal hydration and fetal presentation.
- As per some reports Change of Breech to cephalic presentation has shown increase in AFI.

GJMR-E Classification: NLMC Code: WQ 200



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Severe Oligohydramnios with Good Fetal Outcome- A Case Report

Dr. Sampada ^α & Dr. Tushar Palve ^σ

I. INTRODUCTION

Amniotic fluid provides a protected milieu for the growing fetus, cushioning the fetus against mechanical and biological injury, supplying nutrients and facilitating growth and movement.

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- Other factors noted to affect are maternal hydration and fetal presentation.
- As per some reports Change of Breech to cephalic presentation has shown increase in AFI.

Adverse perinatal outcomes associated with oligohydramnios are – structural anomalies, SGA, umbilical cord compression, uteroplacental insufficiency, which is related to fetal growth restriction, pre-eclampsia and other maternal morbidities and increased incidence of MSAF.

II. CASE REPORT

27 yr female, married since 12 yr, G5P4L2NND2, registered and immunised came to ANC opd with USG obs s/o AFI 0.3 cm at 28 weeks of gestation breech presentation with EFW- 1200 gms.

Targetted AFI was 0+0+0+0.3 cm.

Patient was admitted in ANC ward and evaluated for oligohydramnios. O/E, patient had clinical oligohydramnios with fetal parts easily palpable. Congenital anomaly scan was done which was suboptimal due to advanced gestational age. Patient was given 1 pint alamine with D5. USG obs was done which was s/o SLIUG of MGA 28.3 weeks with AFI- 2cm

with normal doppler with EFW-1272 gms with cephalic presentation.

Adequate intravenous and oral hydration was maintained. Patient was started on cap astymineforte tds, arginine granules tds and protein powder which was continued till delivery. 2 more pint alamine was transfused along with D5.

Serial USG obs with targetted AFI was done, which showed adequate liquor.

- USG obs 10.1.19- s/o SLIUG of MGA 32.4 weeks with adequate AFI – 13-14 cm with normal doppler with EFW- 2035gms with cephalic presentation.
- USG obs 2.2.19- s/o SLIUG of MGA 33.6 weeks with AFI- 12-13 cm with EFW- 2209gms with cephalic presentation.
- USG obs 16.2.19- s/o SLIUG of MGA 36 weeks with AFI- 11-12 cm with EFW- 2798gms with cephalic presentation.

Patient was closely monitored and advised regular ANC visits. FHS/NST monitoring done. DFKC charting was explained to patient. Patient was admitted at 37 weeks of gestation for safe confinement.

A full term male child weighing 2682 kg delivered by emergency LSCS in view of PROM with transverse lie at 38 weeks of gestation. Baby cried immediately after birth with good APGAR score of 8/10 at 1 min and 9/10 at 5 min of birth. Baby was discharged after routine immunisation with uneventful early neonatal period.

III. DISCUSSION

Management depends on the associated pregnancy complication and on the gestational age. when remote from term the endeavour is to prolong the pregnancy with close fetal monitoring.

Nearer term, pregnancy termination is planned with assesment of risk to fetus. Continuous intrapartum fetal heart rate monitoring is offered, if facilities exists, to detect early signs of hypoxia and perform timely intervention.

In our case, oligohydramnios was detected in third trimester but remote from term which was actively managed with alamine infusion, adequate hydration and IUGR regimen. There was change of presentation seen from breech to cephalic in successive scans. Pregnancy was prolonged enough for fetal maturity which resulted in a good fetal and maternal outcome.

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A study "Maternal and fetal outcome in oligohydramnios - study of 100 case" was done from May, 2009 to Nov, 2011, which included 100 patients in 3rd trimester of pregnancy with oligohydramnios. Incidence was seen more in primigravida and operative intervention was also more in primigravida. Most common reason to perform caesarean was fetal distress which was due to coordinate compression and IUGR. Oligohydramnios was related to growth retardation and NICU admission. Approx 7% patients were found with fetoplacental insufficiency on Doppler study.

Another study "Maternal and perinatal outcome in oligohydramnios- study from a tertiary care hospital, Bangalore, Karnataka, India" done from Nov, 2015 to April, 2016 which included 410 pregnant women of gestational age > 37 weeks. Incidence of oligohydramnios was found to be 14%. 62% underwent LSCS with fetal distress being most common indication for it. Incidence of low birth weight was 38.6%. 1/4th of them had APGAR score < 7 at 1 minute. 40% babies were admitted to NICU.

IV. CONCLUSION

- To conclude, oligohydramnios is generally associated with either maternal comorbidities or fetal anomalies, both were not seen in our case.
- Expectant management with maternal hydration, amine infusion etc has comparable maternal and neonatal outcome in women with isolated idiopathic oligohydramnios.



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Alternatives to Fetal Bovine Serum in Different Cell Cultures

By Safaa A. Warda, Hossam A. Fahmy & Fatma F. Warda

Ain shams University

Abstract- Culture of animal cells is a key operation in bioscience at which fetal bovine serum is the most widely used. Other new alternatives were examined for growth of cell culture as newly born calf serum (NBCS), goat serum (GS) and Equine platelet lysate (EPL). Cell growth and viability was investigated in target cells (Vero– MDBK) using different concentration of alternatives. In general the platelet lysate medium 7 -10 % (EPL) supported cell growth and maintained viabilities comparable or superior to (NBCS). Goat serum exhibit heterologous cells in the first passage then the growth were similar to newly born calf serum with adding low concentration % of (GS). - 5% & 6% up to 10% (GS) in MDBK and Vero cells respectively. 0.1% EPL could be used with lower % Goat serum to enhance cellular morphology.

Keywords: equine platelet lysate, goat serum, newly born calf serum, vero, MDBK, cellular viability, serum-free medium.

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Alternatives to Fetal Bovine Serum in Different Cell Cultures

Safaa A. Warda ^α, Hossam A. Fahmy ^σ & Fatma F. Warda ^ρ

Abstract- Culture of animal cells is a key operation in bioscience at which fetal bovine serum is the most widely used. Other new alternatives were examined for growth of cell culture as newly born calf serum (NBCS), goat serum (GS) and Equine platelet lysate (EPL). Cell growth and viability was investigated in target cells (Vero– MDBK) using different concentration of alternatives. In general the platelet lysate medium 7 -10 % (EPL) supported cell growth and maintained viabilities comparable or superior to (NBCS). Goat serum exhibit heterologous cells in the first passage then the growth were similar to newly born calf serum with adding low concentration % of (GS). - 5% & 6% up to 10% (GS) in MDBK and Vero cells respectively. 0.1% EPL could be used with lower % Goat serum to enhance cellular morphology.

Keywords: equine platelet lysate, goat serum, newly born calf serum, vero, MDBK, cellular viability, serum-free medium.

I. BACKGROUND

Fetal bovine serum is the most widely used serum for animal cell culture, mainly due to its high concentration of growth factors and low concentration of immunoglobulins. The use of FBS is associated with challenges such as high variability in batch-to-batch composition, risk of transmitting bovine infections or the initiation of the xenogeneic immune response to bovine antigens and rising cost and complications of the purification of products (Brindley et al., 2012- Bieback, 2013).

Commercially available sera, adult bovine and newly born calf serum (NBCS), sheep, horse, goat sera found to be suitable for the growth of most cell lines and primary culture. Primary cultures from G .pig, chicken embryo fibroblast, monkey kidney (Savant, 1987), peritoneal macrophages were prepared and proliferate in media with goat serum (GS) (Paranjape, 2004). Also, GS used in studies different nutrients & cellular metabolism (Parap, 1995) and virus replication (Paranjape & Cadam 1985). Growth media supplemented with GS were used for in vitro cultivation of Thielaria parasite (Sharma et al., 1998). Developments towards reduced and serum-free media, chemically defined media, maintenance media,

and suspension culture media for anchorage-dependent cell lines have been realized (Tezel and Priore 1998).

A new cell culture supplement, platelet lysate, was evaluated compared with fetal bovine serum (FBS), an established industrial medium for animal cell culture. Generally, platelet lysate medium was less complex than FBS. Platelets considered as concentrated supply of platelet-derived growth factor (PDGF) and transforming growth factor (TGF) (Fukimizu and Grinell 1990) which play an important role in the growth of cell culture as they are participating in many diverse cellular functions. Also, Platelets are the primary source of a number of growth factors, attachment factors (fibronectin and vitronectin), enzymes, serotonin and other factors (Ross and Raines, 1990). The platelet lysate medium demonstrated lack of microorganisms, mycoplasma, and endotoxins (Liselott et al., 2003). There was no significant difference in DNA methylation profiles of Human Platelets lysate and fetal calf serum on mesenchymal cells and did not affect their differentiation potential towards osteogenic or adipogenic lineage (Fernandez-Rebollo et al., 2017)

Thus, the goal of this study, to demonstrate the potential role of newly born calf serum, platelet lysate alone and goat serum, as a growth-promoting supplement in media used for the growth of different cells line culture (Vero & MDBK).

II. MATERIALS AND METHODS

a) Collection of goat blood (According to Paranjape, 2004)

Keep muslin clothes covered bucket & gathered the blood as soon as the animal slaughtered. Poor the blood and leave to clot at room temperature then leave at 4°C overnight. Discard the Cotton muslin, poor the serum and centrifugate 300 rpm for 30 min at 4°C. Filtration by sietzfilter, sterility was applied then the serum was inactivated at 56 °C for 30 min. Quality control testing for the presence of antibodies.

b) Equine growth factors E-GFTM

E-GFTM is a preparation consisting of a pale yellow round cake of Lyophilized equine Platelets Growth Factors E-GFTM According to Schallmoser and Strunk, (2013). Blood (800–900 ml) collected from 5 mature horses; blood centrifuged at 200 g for 15 min. The plasma was then centrifuged at 400 g for 15 min. The platelet pellet then resuspended with aspirated

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platelet-poor plasma. The PC diluted to 1×10^{12} platelets/l after a complete blood platelets count was performed. Platelet lysate then generated by a single freeze/thaw Cycle at 80°C overnight followed by 37°C thaw. Resulting products were pooled, centrifuged at 4000 g for 15 min, and filtered with 0.22 μm filters and was stored frozen at -18°C ready for use. The breakdown of platelets in vitro before lyophilization leads to release of supra-physiological doses of growth factors. The vial is reconstituted in 2 ml of sterile normal saline; gently swirl the vial for 3 minutes just before use in cell cultures. The reconstituted vial shouldn't be used after 1 hr.

c) Cellular toxicity

In 96 wells T. C plate in triplicates wells, dispense $100\mu\text{l}$ of either Vero (African green monkey transformed kidney epithelial cells) and (MDBK) Madien Darby Bovine Kidney cells cell lines supplemented with different concentrations (4, 6, 8, 10, 12 & 15 %) of Growth enhancing additives (GS, EPL, and NBCS). Incubation was performed at 37°C for 2-3 days with daily examination under a light microscope.

d) Cell cultures and Viability

Vero & MDBK cells were cultured in Modified Eagle's Medium (MEM) with 0.1% Penicillin-Streptomycin, in duplicates T.C-flasks for each concentration and incubated at 37°C . Growth promoting additives (GPA) added to media in concentrations 4-10 % goat serum, newly born calf serum and platelet lysate 5-10% for several passages. Typical growth of monolayer of cells was performed. Studying viability of Vero cells after incubation 24 hr., trypsinization applied by Trypsin-EDTA for 1 min cells suspension at 5, 8 & 10% (GEA). Mix the harvested cells with equal volume of Trypan blue stain, counting the cells and viability by improved Neubauer haemocytometer were applied.

$$\% \text{ Viability} = \frac{\text{No. of viable cells}}{\text{Total No. of cell}} \times 100$$

III. RESULTS

a) Cellular toxicity

Vero & MDBK cell lines cultured in medium supplemented with GS (Table, 1) showed No toxicity at all concentrations (6, 8, 10, 12 & 15 %) with the preferable growth rate. Low Concentration of Growth factors at 4% showed abnormal cellular shapes, but these changes did not end with cell death so all tested concentrations can use safely.

The previous cell lines exhibit No toxicity at all concentrations (4, 6, 8, 10, 12 & 15 %) using platelet lysate.

b) Proliferation and viability assays

Goat serum exhibit flattened and irregular heterologous shape of cells in the first passage, after

that adaption of cell lines occurred in the following passages. The results of figure (1) revealed that cells had been proliferated using goat serum in concentration 5% & 6% in MDBK and Vero cells. 0.5-1% Equine Platelet lysate (EPL) could be added to growth media in case of low concentration of (GS) that induced a preferable cellular morphology and proliferation rate. Growth rate% in cell lines recorded 80 and 90% in Vero & MDBK cells respectively at 7% of goat serum.

Figure (3) demonstrated a significant growth rate in either MDBK or Vero cells up to 7- 10% (EPL) supported cell growth and maintained Viabilities comparable or similar to (NBCS). Significant proliferation achieved at 10% in GS, EPL and NBCS.

Table 2 revealed that cell counting and viability of Vero After 24hr cultured in media containing at 8% EPL, NBCS, and GS were 39, 30 and 31 mean while at 10% were 52.2, 47.8

Table 1: Growth factors cytotoxicity on cell culture

Cell line	Concentration of Growth factors %	Affected wells/ Total No.	
		Goat serum	Platelets lysate
Vero and MDBK	4	3/3	1/3
	6	0/3	0/3
	8	0/3	0/3
	10	0/3	0/3
	12	0/3	0/3
	15	0/3	0/3

*No. of wells showed microscopically deviation in cells/total No.

Growth promoting factor	Used%	Vero	MDBK
Goat serum (GS)	4	30	50
	5	50	70
	6	60	70
	7	70	80
	8	90	100
	9	90	100
	10	100	100
(GS) + 1%(EPL)	5	60	70
	6	60	80
	7	80	90
Newly born calf serum. (NBCS)	5	30	50
	6	50	60
	7	60	80
	8	80	90
	9	90	100
	10	100	100

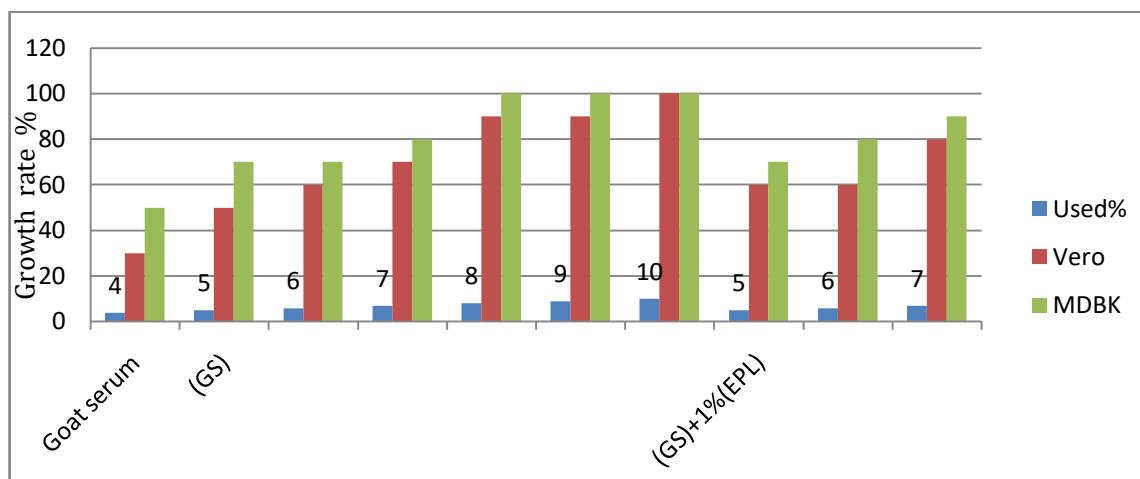


Figure 1: Comparison between GS alone and EPL with GS on the growth of Vero & MDBK cells.

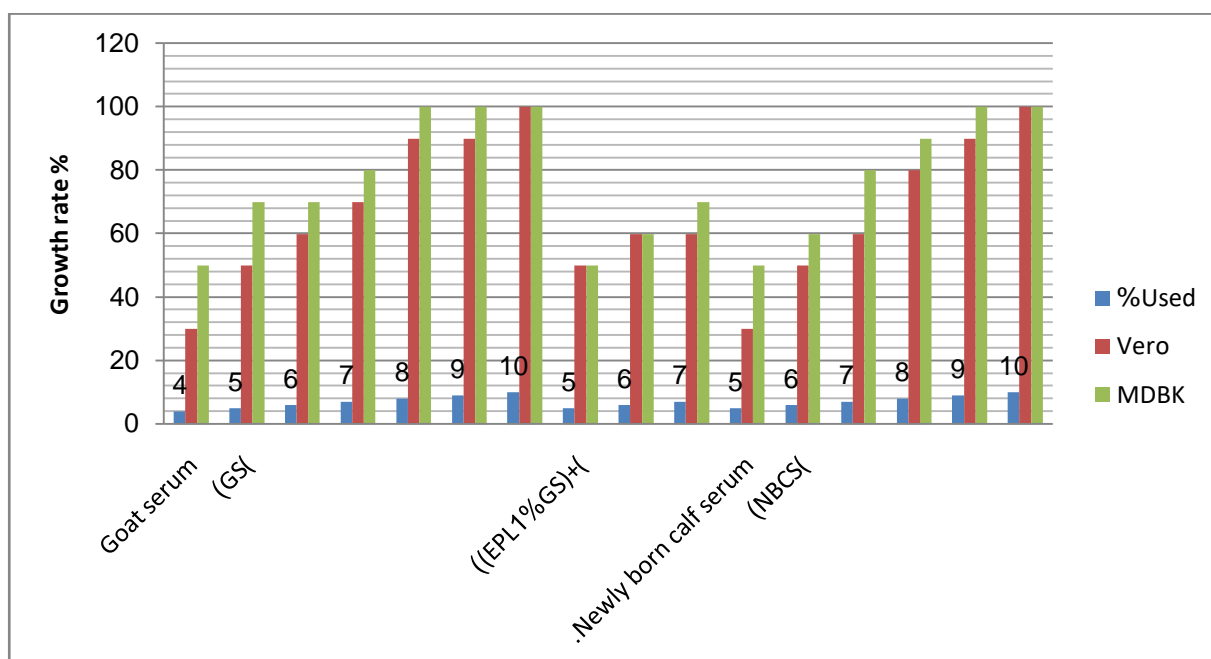


Figure 2: Multiple comparisons in the growth of Vero & MDBK cells using different Growth promoting factors at the same concentrations.

	Used %	Vero	MDBK
Newly born calf serum (NBCS)	5	30	50
	6	50	60
	7	60	80
	8	80	90
	9	90	100
	10	100	100
Equine platelet lysate (EPL)	5	30	30
	6	50	60
	7	60	80
	8	80	90
	9	90	100
	10	100	100

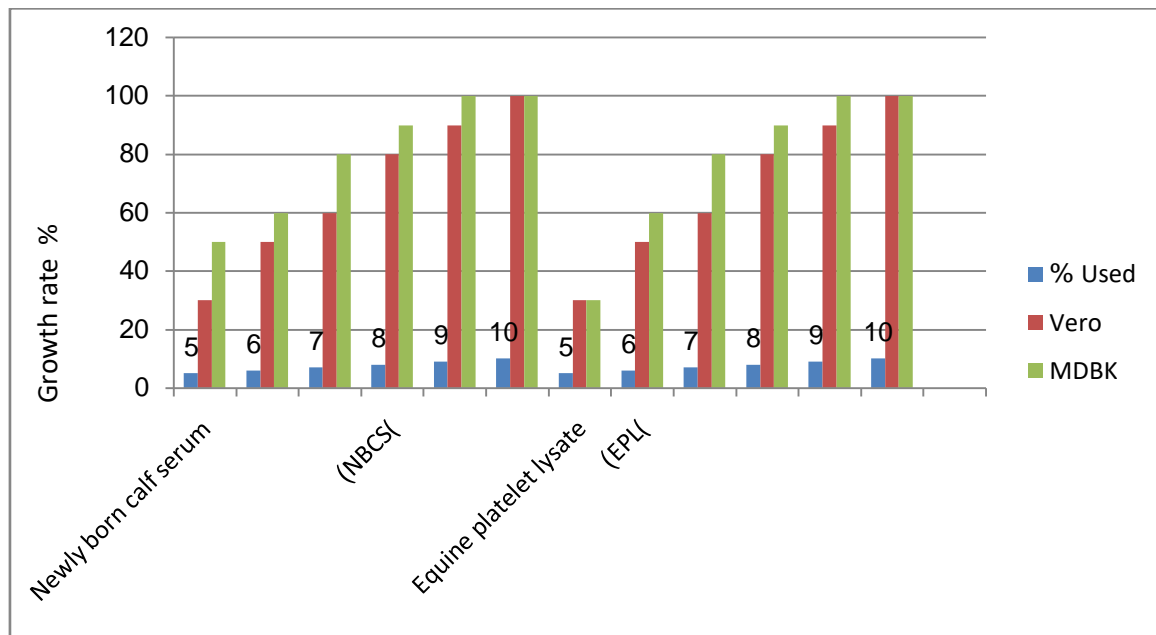


Figure 3: Comparison between NBCS and EPL on the growth of Vero & MDBK cells.

Table 2: Cell counting and viability of Vero cells Cultured in platelet lysate, newly born calf Serum and goat serum

Growth promoting factors	Used %	Viable cells/1ml	died cells/1ml	Total No.	Viability %
Equine platelet lysate (EPL)	5	2.35×10^4	1.35×10^5	1.58×10^5	14.8
	6	9.85×10^5	3.18×10^5	5.12×10^6	19
	8	2.05×10^6	4.13×10^5	5.13×10^6	39
	10	3.88×10^6	3.54×10^6	7.42×10^6	52.2
Newly born calf Serum (NBCS)	5	2.3×10^4	1.78×10^5	4.69×10^4	12.9
	8	2.05×10^6	6.87×10^6	5.78×10^6	30.2
	10	3.79×10^6	7.92×10^6	6.90×10^6	47.8
Goat serum(GS)	5	2.8×10^3	1.85×10^5	2.16×10^5	13
	8	2.13×10^6	4.05×10^6	5.98×10^6	31
	10	3.99×10^6	4.38×10^6	7.57×10^6	47.6

IV. DISCUSSION

Culture of animal cells is key operation in bioscience whether it is related to research at universities or industrial production of pharmaceuticals with the help of gene technology (Hodgson, 1995). Fetal bovine serum (FBS) usually considered as the standard gold serum as a supplement for growth of cell lines. Considerable efforts have been made to reduce or eliminate serum proteins or even using its alternatives as newly born calf serum (NBCS), goat serum (GS) and Equine platelets lysate (EPL) as a serum-free media.

In this work, the first plan was establishing another alternatives to fetal bovine serum by using (NBCS) and (GS). Medium supplemented with (GS) could be used in the propagation of Vero & MDBK cell lines. Blind 7 and 4 successive passages were applied in Vero& MDBK cell lines respectively. figure (1) revealed that cells have been proliferated using goat serum alone compared to GS beside EPL or NBCS (figure, 2) cell growth was achieved in media supplemented with (GS) at concentration 5% & 6% up to 10% in MDBK and Vero cells. Another point of view, the percentage of (GS) could be decreased when 0.5-1% (EPL) was added to

growth media. This also achieved a preferable cellular morphology and proliferation rate. The growth rate was higher in GS than NBCS at similar concentration up to 5-8% thereafter become the same. All growth promoting factors were significantly increased at 10%.

This finding coincidence with the result of Paranjape, 2004 that 10 % goat serum containing media is similar to fetal serum in many cell line and primary cells except BHK-21 cell line. The author found the relation between DNA, total protein and cell count of Vero cells propagated by using 10% (GS).

Furthermore, the author added that Vero cells adapted & maintained on (GS) were used in preparation of CFT & detection of Dengue virus antigen from clinical samples & interferon production in LM & MFS cells.

The second plan was designated to find alternative use to FBS by (EPL) as a serum free-media .the results in (figure, 3) demonstrated significant growth rate in either MDBK and Vero cells up to 7- 10%. The proliferation rate of EPL was nearly similar to NBCS proliferation and cellular viability is dose dependent.

The number of cells correlated with (EPL) % in the supplemented medium that agree with Liselott et al., 2003, concluded that 10% EPL induces cell growth, viability and product formation using a number of target cells including myelomas, hybridomas, hepatocytes, fibroblasts and epithelial cells.

Also Doucet et al., 2005 and Horn et al., 2010 have been used (PL) as an FBS substitute in expansion medium (EM) to support the growth of human mesenchymal cells that induced higher mitogenic effect than fetal bovine serum (FBS).

Concerning with the counting and viability of adherent Vero cells after 24hr incubation at 37 °C using (GS), NBCS and (EPL) in table 2, observed that (EPL) supplemented media induce the best viability for cell culture. There was no apparent difference in Viability between (NBCS) and (GS) in spite of higher died cells in (NBCS) supplemented media.

The result disagrees with Russell & Koch, 2015, recorded no significant difference between the pooled (EPL) and (FBS) treatments in mesenchymal (MSC) cells up to a concentration of 30%.

V. CONCLUSION

A useful alternative to fetal bovine serum in the medium for animal cell culture as a growth-promoting factor could be achieved by using:

1. 5% & 6% up to 10%- (GS) in MDBK and Vero cells respectively.
2. (GS) % could be decreased when 0.5-1% Equine Platelet lysate (EPL) added to growth medium.
3. 7-10% newly born calf serum.
4. Up to 7 -10 % (EPL) alone Perspective not only economic view that produced in large amounts at relatively low cost but also hygienic serum-free

medium for cell cultures to avoid the risk of transmitting bovine infections.

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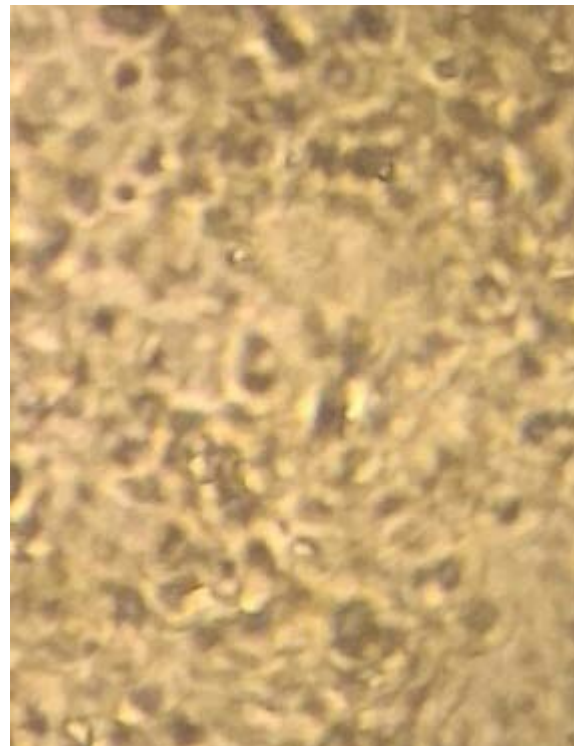


Photo 1: MDBK cells cultured in 10% EP (10x)



Photo 2: MDBK cells cultured in 10% EP (4x)

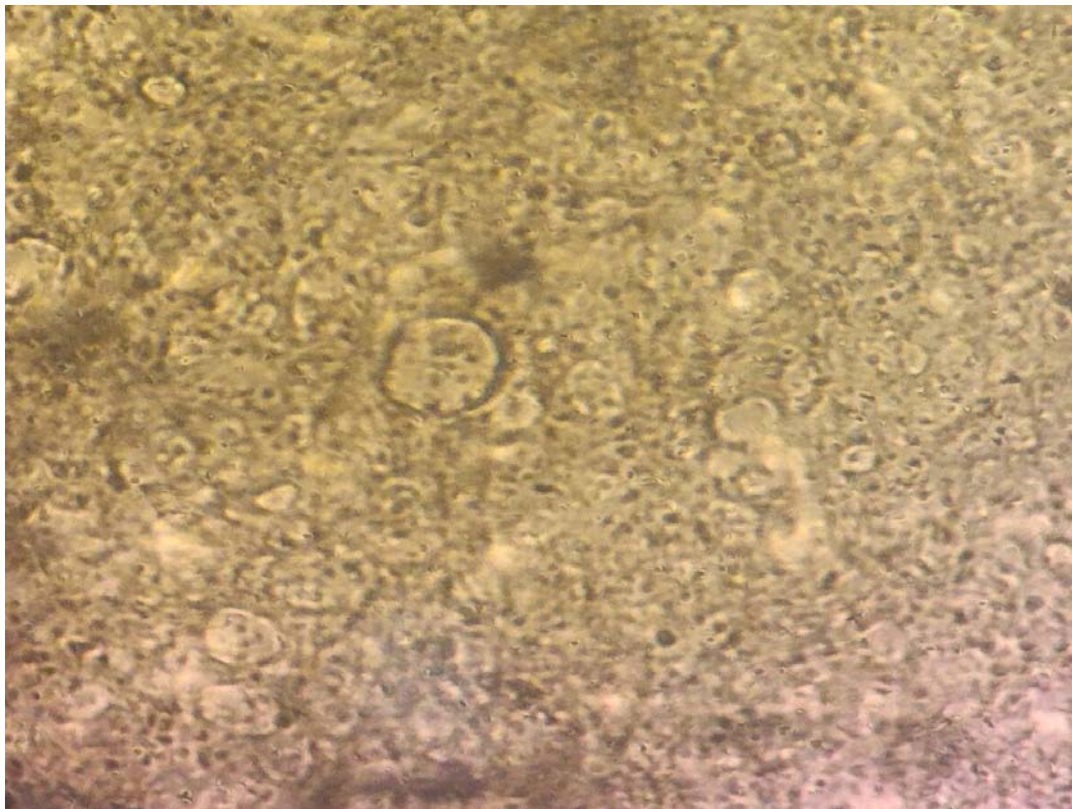


Photo 3: MDBK cell line cultured in 5% growth-promoting factor (10x)

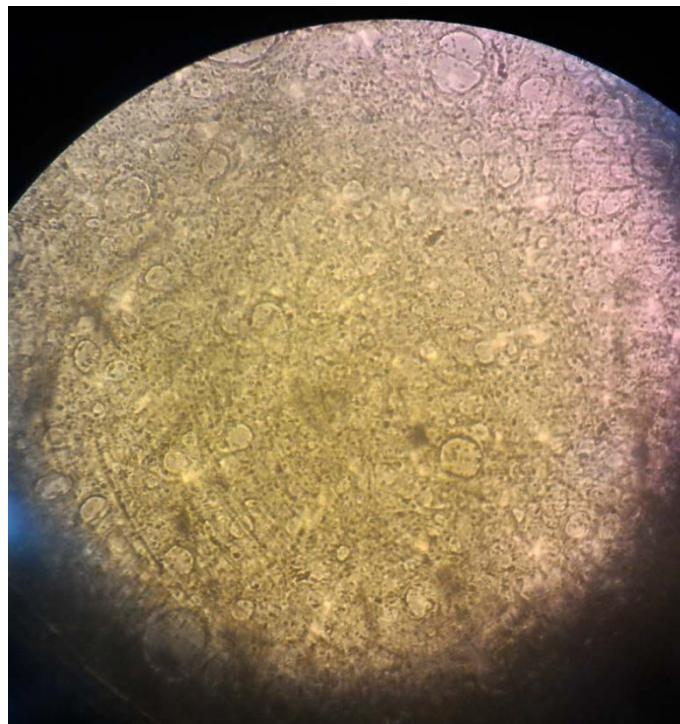


Photo 4: MDBK cell line cultured in 5% growth-promoting factor (4x)

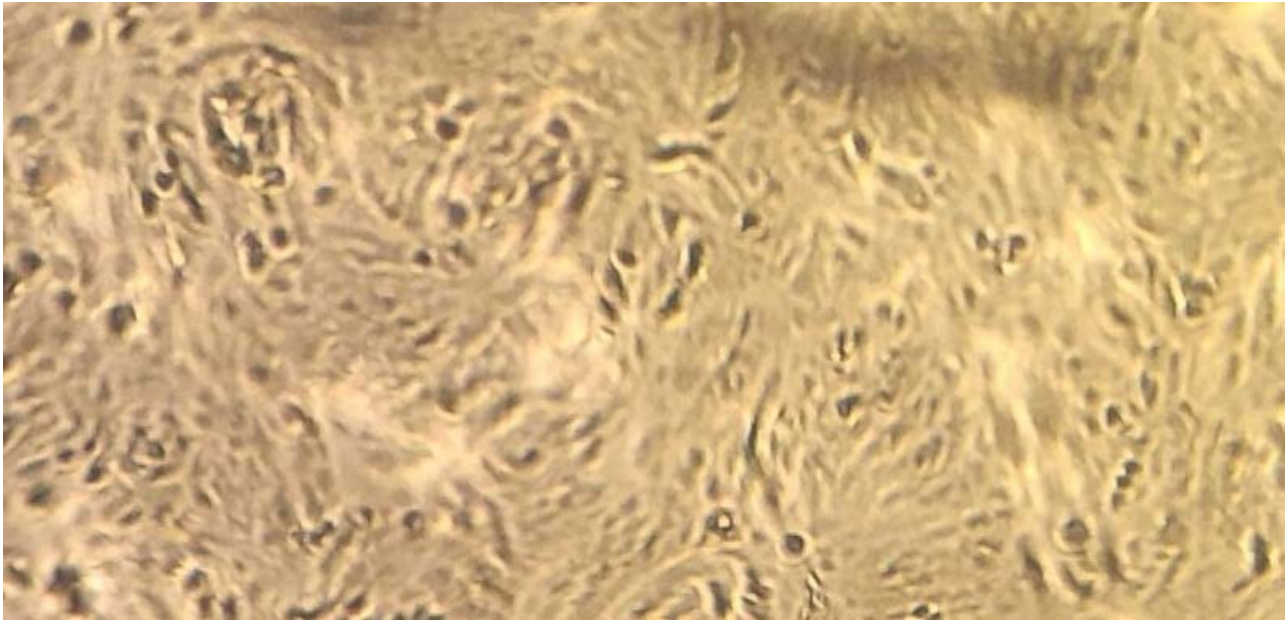


Photo 5: Vero cell line cultured in 10% growth-promoting factor 10x

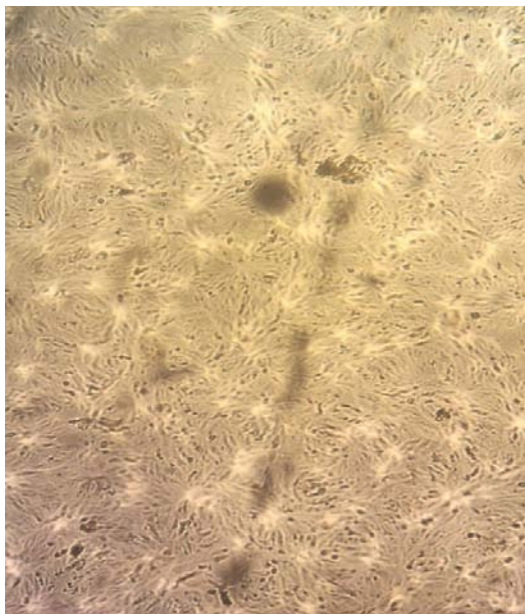


Photo 6: Vero cell line cultured in 5% growth promoting factors

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Keywords: health education, randomization, reproduction, sexual behaviors, undergraduates.

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Reproductive Health Education Package to Reduce Risky Sexual Behaviors among Undergraduates in Selected State Universities in Sri Lanka: A Controlled Trial

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Results: The reproductive health package reduces the RSB at three months of follow up after the intervention although it was not statistically significant (AOR=0.80; 95% CI 0.28- 2.31). Further, the package significantly improves the knowledge (AOR=11.75; 95% CI 7.04- 19.63) and desirable attitudes towards reproductive health (AOR=6.13; 95% CI 3.64- 10.32).

Conclusions: The educational intervention may reduce RSB after three months. It was effective for the improvement of good knowledge and desirable attitudes on reproductive health.

Keywords: health education, randomization, reproduction, sexual behaviors, undergraduates.

I. PLAIN ENGLISH SUMMARY

Risky sexual behaviors are behaviors leading to unwanted pregnancies and sexually transmitted infections. This research was done to assess the success of the developed package to lessen the risky sexual behaviors. A health education package was

developed with the help of available packages from other countries to suit for young adults in Sri Lanka. Then the package was introduced to a group of selected undergraduates in two universities in the Western province of Sri Lanka. Before the introduction of the package, their level of risky sexual behavior has been assessed with a paper of questions. After three months of the introduction of the package, the risk level was assessed again. The results revealed that the educational intervention may reduce risky sexual behaviors. It also revealed the improvement of the knowledge and positive attitudes on risk behaviors and reproductive health.

II. INTRODUCTION

Risky sexual behaviors (RSB) are behaviors leading to unintended pregnancies and sexually transmitted infections (STI) as defined as Centre for Disease Control (CDC) [1]. Three studies reported that the prevalence of risk behavior was higher than the level expected by parents and teachers [2, 3, 4]. Being in the age groups of adolescents and young adults, undergraduates may not be spared in practicing RSBs. Adolescents and young adults need different kinds of programs to prevent RSB. There is inconsistency among youth with respect to cognitive and social maturity and sexual experience. Therefore, interventions need to be tailor-made to meet the different requirements of different youth groups [5]. One review showed that determinants of safer sex behaviors among college students were alcohol usage, religiosity, barriers to condom use and perceived social norms. [6] Sexuality education has often concentrated on the risks associated with sexual behavior such as unintended pregnancies and STIs. Interventions for young people are usually implemented in schools, through peer educators, as community activities, and as mass media campaigns.

Several studies were performed to aim the reduction of RSB among adolescents. Two quasi-experimental studies have proven that educational interventions were effective in improving knowledge in

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reproductive health (RH) in youth [7, 8]. Another trial, which was conducted among adolescent girls between 15 to 19 years of age in India, revealed significant improvement of knowledge at one month after implementation of a RH package. [9]. Another quasi-experimental intervention targeted for adolescents of schools in China showed that an educational intervention is effective for increasing knowledge and improving attitude, not the behavior [10]. Similarly, a cluster randomized trial which was carried out to assess theoretically based sex education programme conducted by teachers revealed that the intervention did not reduce sexual risk taking in school going adolescents [11]. In contrast, another cluster randomized trial reported that in addition to sex education, skills building and motivational intervention was effective for reducing RSB among African-American male adolescents [12]. A review reported that sexual health education programs based on a comprehensive curriculum are able to improve knowledge and lessen the RSB [13]. It further reported that sex education programs do not increase sexual activity among adolescents and young people. Results from a Cochrane review showed that a combination of educational and contraceptive-promoting interventions showed the reduced the risk of unintended pregnancies among adolescents [14]. A study carried out to assess the relationship between condom availability and RSB among adolescents in high schools in Massachusetts revealed the success of programmes in human immunodeficiency virus (HIV) prevention [15].

Promoting Sexual Health is a comprehensive curriculum designed to provide information and build skills that reduce unintended pregnancies, reduce the transmission of STI including HIV and improve the quality of sexual experience [16]. The curriculum was designed for young adults in ages 18–24 years who are or to be sexually active to promote sexual health by addressing several aspects of sexuality such as the positive side of sexuality, pleasure, and satisfaction. Further, it is based on a logic model that specifies the important goals, particular behaviors that will lead to those health goals, and cognitive, sexual, psychosocial factors that affect those behaviors. Several authors have used similar contents within their interventions to reduce RSB [8, 9, 10, 11, 15, 17].

There is a dearth of published literature on interventions for risky sexual behavior in Sri Lanka. Hettiarachchi has developed and successfully delivered a new educational intervention for teachers to increase the knowledge and attitude in sexual and RH among school children and teachers in Sri Lanka [18]. There are cultural taboos that have made it difficult for parents, teachers, and community leaders to openly discuss sexual issues among themselves or with adolescents [19]. The same cultural taboos that have hampered

even marketing campaigns to promote greater use of contraceptives including condoms. This emphasizes the need for culturally sensitive reproductive education package for reducing RSB. Only those students who perform well in advance level examination are selected for state universities in Sri Lanka. Most of the university students stay away from their families. They are young adults and have more independent than school going adolescents. Therefore the university students are more vulnerable than the school children. Findings of this study are intended to inform the relevant authorities to design and deliver risk behavior reducing program for the youth in universities. Therefore, the objective was to assess the effectiveness of an educational intervention to reduce RSB among undergraduates in the selected state universities in Sri Lanka.

III. MATERIALS AND METHODS

A quasi-randomized controlled trial was conducted among the second and third year undergraduates in the University of Kelaniya and the University of Sri Jayewardenepura in 2014.

The exclusion criteria were undergraduates from foreign countries due to their different socio-cultural background and clergymen undergraduates due to the sensitive nature of the selected subject.

The intervention was a health education intervention conducted by four medical officers. It consisted of two-day interactive sessions of three and a half hours, conducted one week apart. No educational sessions were conducted to the control group. The intervention was designed using the curriculum on 'Promoting Sexual Health' [16]. Expert opinion was sought from the National STD/AIDS control programme and Health Education Bureau (HEB) for adapting the curriculum. The component which aim to improve the quality of sexual experience were removed. Case studies were changed according to local culture. The baseline information on knowledge, attitudes, and behaviors on reproductive health from previous national survey was utilized for the modification of the intervention. The available 'information, education and communication' (IEC) materials on family planning, STI and RSB were used with the expert opinion for the development of new materials. The developed intervention covered relevant topics in the prevention of STIs and unintended pregnancies. It included facts on contraceptive methods, i.e. effectiveness, side effects and ways of overcoming barriers. Furthermore, it described STIs with possible consequences and informs about places for testing. Dual protection given from condom was highlighted and condom demonstration was included emphasizing the problems in use. Multiple and overlapping partners' role in STIs was discussed. A mutual monogamous relationship was encouraged and the importance of the ability to insist on condom

use was emphasized. In addition developed self-efficacy and skills to express or refuse sex. Almost all aspects in the prevention of STI and unintended pregnancies were covered by the developed intervention. The developed intervention was pretested among a group of 20 undergraduates outside the study population to test the feasibility, accessibility, co-operation and other logistical aspects of the designed intervention. Necessary changes were made to the intervention accordingly.

The primary outcome was the reduction of RSB among undergraduates during the three months of post-intervention period. The secondary outcomes were overall knowledge and attitudes on RH among undergraduates at three months after the intervention. RSB is defined as a practice of one or more following behavior/s, having more than one sexual partner, alcohol use with sexual activities, inability to use condoms to prevent STI with commercial sex workers or non-commercial partners and inability to use contraceptives in sexual activities with commercial sex workers or non-commercial partners. The overall knowledge on reproductive health was based on the knowledge on unsafe abortions (7 statements), contraceptives (10 statements), condoms (7 statements), STIs (28 statements) and HIV/AIDS (13 statements). One mark was given for each correct answer and zero marks was given for incorrect and do not know answers. A composite score on knowledge ranged from 0 to 65 and expressed as percentages. Equal or more than 75% was categorized as good knowledge and less than 75% as average knowledge for data analysis. The overall attitudes were based on attitude towards the use of contraceptives (5 statements), condoms (8 statements) and HIV/AIDS (9 statements). Each statement comprised of five responses as strongly agree, agree, neutral, disagree, strongly disagree. One mark was allocated for desirable attitudes while minus one mark for undesirable attitudes and zero marks for neutral responses. The range was from (-22) to +22. To make the values positive, +22 was added. The composite score ranged from 0 to 44. Cutoff marks were given for desirable and undesirable attitudes according to the inter quartile range. Those who scored 75th centile or above were taken as desirable attitudes and those who scored below 75% were taken as undesirable attitudes.

The University of Kelaniya and the University of Sri Jayewardenepura were selected as the intervention group and the control group respectively by tossing a coin. Ten clusters were selected randomly from all the clusters in each university. A cluster was defined as a tutorial group or a whole batch according to the structure of the selected undergraduate's group. We expected that the intervention will reduce the RSB from 13% to 6% among undergraduates [4]. Therefore, the

calculated sample size was 301 for each group considering power as 80% and a false positive rate of 5%.

The developed intervention was introduced with IEC materials and all sections were discussed in detail. The opportunities were given to clarify any doubts. The intervention was conducted in the lecture halls for ten groups of undergraduates. Informed written consent was taken from the participants. A pre-tested self-administered questionnaire was used to assess pre and post-intervention data. The questionnaire comprised of five sections, the section I on socio-demographic and economic characteristics of the undergraduates, section II on other risk behaviors including alcohol, smoking and illicit narcotic drugs, section III on knowledge in selected aspects of RH, section IV on attitudes in selected aspects of RH, and section V on sexual behavior. The validity of the questionnaire was ensured by assessing the face, content and consensual validity. Content validity was assessed by checking whether or not all aspects of measures were covered using literature review and expert opinion. Consensual validity was determined by assessing the agreement of the experts on whether or not the conceptual definition has been used appropriately in the tool. A multi-disciplinary panel of experts in the fields of public health and reproductive health was used for assessment of validity. On the data collection day, the selected undergraduates (the tutorial group or batch which was identified) were taken to a separate lecture hall. The participants were seated with appropriate gaps from each other to maintain confidentiality. An envelope was given to each participant to hand over the filled questionnaire.

Baseline data between the intervention and the control groups were analyzed using the chi-square test. The effectiveness of the intervention was assessed by comparing post-interventional data between intervention and control groups, the results were expressed as odds ratios (OR) and its 95% confidence intervals (CI). Baseline imbalances were controlled by applying multiple logistic regression. Sensitivity analysis was performed assuming that all participants who did not complete the follow-up data in the intervention group had high risk of RSB, average knowledge and undesirable attitudes, and those who were in the control group had a low risk of RSB, good knowledge and desirable attitudes.

IV. RESULTS

Of 309 undergraduates who were eligible and invited, only 300 post-interventional data could be collected from the intervention group. Of 427 undergraduates who were eligible and invited in the control group, 297 participated in the post-interventional assessment.

There were no statistically significant differences in the intervention and the control groups with respect to sex, ethnicity, religion, age and marital status (Table 1).

The proportion of undergraduates staying at boarding places were higher among the control group than the intervention group ($p < 0.001$). The control group had more undergraduates with family income $\leq 50,000$ rupees per month than the intervention group ($p = 0.014$). There were more undergraduates in Bio-Science stream in the intervention group (35.9%) than the control group (7.0%) ($p < 0.001$). With regard to school type, the control group consisted of 35.9% from mixed schools (both girls and boys) compared to 44.6% in the intervention group ($p = 0.04$). There were no differences with regard to the importance of religion and participating in religious activities between intervention and control groups. With regard to the availability of relatives and friends to discuss sexually related issues, there was no difference between the groups.

Significant differences were observed between the intervention and the control groups with regard to some risk behaviors before the intervention. Attended nightclub ever in life, usually going to cinema halls, taken alcohol ever and using internet > 2 hours per day showed significant differences between the two groups. (Table 2)

The undergraduates in the intervention group reported a higher percentage of good knowledge on contraceptives ($p < 0.001$) than the control group (Table 3).

Knowledge on condoms, knowledge on HIV/AIDS, knowledge on STIs and overall knowledge on

RH did not show significant differences between the two groups. There were no differences with regard to attitudes in contraceptives, condoms, HIV/AIDS or overall attitudes in RH among undergraduates between the intervention and the control groups (Table 3 and 4). There was no significant difference of RSB within last three months between the intervention and the control group before the intervention (Table 4).

There was no significant difference observed between the intervention and the control group after three months of intervention with related to RSB (OR=1.39, 95% CI 0.81-2.39) (Table 4). However the adjusted OR was 0.8 (95% CI 0.28-2.31) which indicate a lower risk of RSB of the intervention group even though it was not statistically significant.

Overall good knowledge on reproductive health was higher (52.2%) among the intervention group compared to the control group (12.8%) at three months after the intervention (OR=7.48, 95% CI 4.97 -11.26) (Table 4). Even after adjustment for baseline imbalances, it remained higher (adjusted OR=11.75; 95% CI 7.04-19.63). The overall positive attitudes towards RH among undergraduates was higher in the intervention group (50.7%) compared to the control group (26.9%). The difference was significant statistically (OR=2.79, 95% CI 1.98 -3.92) (Table 4). Even after adjustment for baseline imbalances it was higher (adjusted OR = 6.13; 95% CI 3.64-10.32). The sensitivity analysis has not changed the direction of the association with any outcome (Supplementary Table 1).

Table 1: Socio-demographic, economics and other basic characteristics among intervention group and control group

Variable		Intervention N=309 n (%)	Control N=427 n (%)	χ^2 p value
Sex	Male	87 (28.2)	127 (29.7)	0.22
	Female	222 (71.8)	300 (70.3)	0.64
Ethnicity	Sinhalese	306 (99.0)	399 (93.4)	13.9
	Muslim	3 (1.0)	28 (6.6)	< 0.001
Religion	Buddhist	290 (93.9)	388 (90.9)	2.2
	Non Buddhist	19 (6.1)	39 (9.1)	0.14
Resident ¹	Boarding place	197 (63.8)	332 (77.9)	17.8
	Home/Relative	112 (36.2)	94 (22.1)	< 0.001
Age ²	≤ 22 years	99 (32.1)	125 (29.5)	0.59
	≥ 23 years	209 (67.9)	299 (70.5)	0.44
Marital status ¹	Unmarried	305 (98.7)	418 (97.9)	0.68
	Ever married	4 (1.3)	9 (2.1)	0.41
Family income (Rs.) ³	< 50000	242 (84.3)	363 (90.5)	6.1
	> 50000	45 (15.7)	38 (9.5)	0.014
Financial assistance (Rs.) ⁴	< 3000	200 (92.2)	343 (93.0)	0.12
	> 3000	17 (7.8)	26 (7.0)	0.72
Academic year	Second	192 (62.1)	241 (56.4)	2.4
	Third	117 (37.9)	186 (43.6)	0.12
Study stream	Non-Biology	199 (64.4)	397 (93.0)	95.0
	Biology	110 (35.6)	30 (7.0)	< 0.001

School type ⁵	Non-mixed	104 (44.6)	112 (35.9)	4.26
	Mixed	129 (55.4)	200 (64.1)	0.04
Importance of religion	Very important	257 (83.2)	350 (82.0)	0.18
	Not very important	52 (16.8)	77 (18.0)	0.67
Participating religious activities ⁶	Weekly or more	141 (45.9)	205 (48.6)	0.50
	Less than weekly	166 (54.1)	217 (51.4)	0.48
Access to a relative to talk in sexual issues	Yes	238 (77.0)	315 (73.8)	1.02
	No	71 (23.0)	112 (26.2)	0.31
Access to a friend to talk in sexual issues	Yes	266 (86.1)	384 (89.9)	2.57
	No	43 (13.9)	43 (10.1)	0.11

¹missing data= 1, ² missing data= 4 ³missing data =48, ⁴missing data=150 ⁵missing data=191 ⁶missing data=7

Table 2: Baseline risk behaviors among undergraduates by the intervention Group and the control group

Variable	Intervention N=309 n (%)	Control N=427 n (%)	χ^2 p value
Attend night clubs*			
Yes	12 (3.9)	4 (0.9)	7.25
No	297 (96.1)	420 (99.1)	0.007
Attend nightclubs in previous one month			
Yes	6 (1.9)	3 (0.7)	2.28
No	303 (98.1)	424 (99.3)	0.13
Using Internet facilities			
Yes	290 (93.9)	378 (88.5)	6.07
No	19 (6.1)	49 (11.5)	0.014
Using Internet facilities >2 hours per day			
Yes	105 (34.0)	95 (22.2)	12.46
No	204 (66.0)	332 (77.8)	<0.001
Going to cinema halls*			
Yes	187 (60.5)	219 (51.5)	5.85
No	122 (39.5)	206 (48.5)	0.02
Going to cinema halls in last month			
Yes	30 (9.7)	27 (6.3)	2.88
No	279 (90.3)	400 (93.7)	0.09
Had taken alcohol ever			
Yes	85 (27.5)	76 (17.8)	9.89
No	224 (72.5)	351 (82.2)	0.002
Had taken alcohol in last three months			
Yes	48 (15.5)	42 (9.8)	5.42
No	261 (84.5)	385 (90.2)	0.02
Had smoked ever			
Yes	33 (10.7)	30 (7.0)	3.06
No	276 (89.3)	397 (93.0)	0.08
Had smoked in last three months			
Yes	18 (5.8)	19 (4.4)	0.71
No	291 (94.2)	408 (95.6)	0.39
Had taken Cannabis ever			
Yes	15 (4.9)	13 (3.0)	1.61
No	294 (95.1)	408 (95.6)	0.21
Had taken Cannabis in last three months			
Yes	4 (1.3)	5 (1.2)	0.02
No	305 (98.7)	422 (98.8)	0.88
Had physical fighting in last year			
Yes	14 (4.5)	16 (3.7)	0.28
No	295 (95.5)	411 (96.3)	0.59
Had physical fighting in university life			
Yes	18 (5.8)	21 (4.9)	0.29
No	291 (94.2)	406 (95.1)	0.59

*missing data=3 **missing data=2

Table 3: Baseline knowledge and attitude on reproductive health among the undergraduates by the intervention and the control Group

Knowledge/Attitude	Intervention N=309 n (%)	Control N=427 n (%)	χ^2 p value
Contraceptives			
Good knowledge	112 (36.2)	88 (20.6)	22.15
Average Knowledge	197 (63.8)	339 (79.4)	<0.001
Condoms			
Good knowledge	126 (40.8)	156 (37.2)	0.95
Average Knowledge	183 (59.2)	268 (62.8)	0.33
STI			
Good knowledge	42 (13.6)	62 (14.5)	0.13
Average Knowledge	267 (86.4)	365 (85.5)	0.72
HIV/AIDS			
Good knowledge	172 (56.2)	244 (57.4)	0.10
Average Knowledge	134 (43.8)	181 (42.6)	0.75
Contraceptives			
Desirable attitude	85 (27.5)	113 (26.5)	0.10
Undesirable attitude	224 (72.5)	314 (73.5)	0.75
Condoms			
Desirable attitude	92 (29.9)	140 (32.8)	0.71
Undesirable attitude	216 (70.1)	287 (67.2)	0.40
HIV/AIDS			
Desirable attitude	127 (41.1)	174 (40.9)	0.002
Undesirable attitude	182 (58.9)	251 (59.1)	0.96

¹missing data=5, ²missing data=1, ³missing data=2

Table 4: Risky sexual behavior, knowledge and attitude on reproductive health among undergraduates' pre and post-intervention

Outcomes	Pre-intervention			Post-intervention			
	Intervention n group N=309	Control group N=427	OR 95 % CI p value	Intervention n group N=300	Control group N=297	OR 95 % CI p value	AOR 95 % CI p value
Risky sexual behavior			1.36			1.39	0.8 *
Yes	48 (15.5)	51 (11.9)	(0.89-2.1)	34 (11.3)	25 (8.4)	0.81-2.39	0.28-2.31
No	261 (84.5)	376 (88.1)	0.16	266 (88.7)	272 (91.6)	0.23	0.68
Overall knowledge on reproductive health			2.02			7.48	11.75 **
Good knowledge	63 (20.4)	48 (11.2)	1.34- 3.0	157 (52.3)	38 (12.8)	4.97- 11.26	7.04-19.63
Average knowledge	246 (79.6)	379 (88.8)	<0.001	143 (47.7)	259 (87.2)	<0.001	<0.001
Overall attitudes in reproductive health			1.04			2.79	6.13 ***
Desirable attitudes	79 (25.6)	106 (24.8)	0.74-1.46	152 (50.7)	80 (26.9)	1.98-3.92	3.64-10.32
Undesirable attitudes	230 (74.4)	321 (75.2)	0.82	148 (49.3)	217(73.1)	<0.001	<0.001

OR odds ratio, AOR adjusted odds ratio

* Adjusted for pre-intervention RSB, attended night club in last one month, reside outside the home and bio-science stream.

** Adjusted for pre-intervention knowledge on reproductive health, reside outside the home and bio-science stream.

*** Adjusted for pre-intervention attitudes on reproductive health, pre-intervention knowledge on reproductive health and bio-science stream.

V. DISCUSSION

The intervention was not effective for reducing RSB at three months after the intervention. The overall knowledge and desirable attitudes on RH had been improved significantly in the intervention group when

compared to the control group after three months of the intervention.

The two groups were allocated to the intervention and control group randomly to minimize selection bias. Contamination effect was minimized by selecting two separate universities for the intervention

and control groups. There are only four universities in the western province, therefore considering a university as a cluster is not adequate for a cluster randomized trial. We randomly selected 10 clusters from each university. Even though we did not consider cluster effect for the calculation of sample size and statistical analysis. It was not feasible to conduct a cluster randomized trial within a university due to a risk of contamination. Some of the published results from RH interventions based on quasi-experimental studies [7, 8, 9, 10]. It is natural to have a higher rate of loss to follow up among the control group as they were not intended for any benefits. The selection of three months of follow up period was done according to available literature and considering the feasibility of the collection of post-interventional data [12]. However, one study reported that the time period of three months may not be adequate to detect a significant reduction of RSB [9]. Further, the undergraduates in our study were a low-risk cohort and therefore the usefulness of the intervention is limited. However, the intervention maybe effective for more vulnerable adolescents who have fallen out of the education system. The impact of the un-blindness of the investigators did not affect the outcome assessments as the use of a self-administered questionnaire. Further, the blindness of the participants was not feasible for health educational intervention. After controlling the baseline imbalance between the intervention and the control group, changed the effect of the intervention from harmful effect to a protective effect for RSB. However, it was not statistically or practically significant.

In contrast to our findings, a similar type of health education intervention carried out among male adolescents in America has improved RSB among them [12]. Even though the follow-up period was similar they have used different IEC materials such as videos, games, exercises and role-playing. The observed difference of results in the present study could be due to methodological variations, the difference in intervention curriculum and the baseline characteristics of the study population. A review of sexual education interventions to assess the impact on sexual behaviors revealed that sex education programmes were effective at reducing RSB among adolescents and young people [13]. The review revealed the effectiveness of reducing the number of sexual partners, increasing condom usage and increasing contraceptive usage.

Reproductive health-related information can be delivered successfully by health education interventions in various academic institutions through various channels. Knowledge and attitudes were improved among school-going adolescents by delivering a health educational intervention directed at school teachers in a district of Sri Lanka [18]. It represented a different study population; school children in Grade nine were educated through their teachers. Knowledge and

attitude improvement were observed among them and their evidence supports the findings of the present study. Another health educational intervention among female students between 15 to 19 years of age carried out in India, reported improvement of knowledge in reproductive health after one month of post-intervention [9].

VI. CONCLUSIONS

The educational intervention may reduce RSB after three months. It was effective for the improvement of good knowledge and desirable attitudes on reproductive health. Our study shows that educated youth improved their knowledge and attitudes about reproductive health, which is not surprising with their educational backgrounds. The intervention can be applied even for youths in working places without many alterations. However further research is needed to assess whether RSB for this group is improved in the long term follow them up.

VII. LIMITATIONS

Follow up period after the intervention was three months period which may be inadequate to see significant changes in behavior. Even though it was unfeasible, unblinding of the participants might underreport of their self-assessed RSB.

Declaration

Ethics approval and consent to participate: Ethical clearance was taken from the Ethical Review Committee, Faculty of Medicine, University of Kelaniya. Administrative clearance was obtained from the Vice Chancellors and Deans of the selected faculties. Informed written consent was obtained from the participants.

Consent for publication: Not applicable.

Availability of data and material: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests: The authors declare that they have no competing interests.

Clinical Trial registry: SLCTR/2014/003, registered on 1st of Feb 2014

Funding: The Medical Research Institute of the Ministry of Health, Sri Lanka funded for the data collection of the study.

Authors' contributions: Both authors have contributed equally to the design the study. UP and CA analyzed and interpreted the data. UP was responsible for the conduct of the literature review and implementation of study and a major contributor in writing the manuscript. All authors read and approved the final manuscript.

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List of Abbreviations

CDC: center for disease control.

CI: Confidence intervals.

HIV: Human immunodeficiency virus.

OR: Odds ratio.

RH: reproductive health.

RSB: Risky sexual behavior.

STI: sexually transmitted infections.

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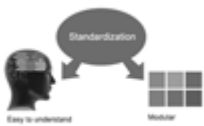
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11. Pick a good study spot: Always try to pick a spot for your research which is quiet. Not every spot is good for studying.

12. Know what you know: Always try to know what you know by making objectives, otherwise you will be confused and unable to achieve your target.

13. Use good grammar: Always use good grammar and words that will have a positive impact on the evaluator; use of good vocabulary does not mean using tough words which the evaluator has to find in a dictionary. Do not fragment sentences. Eliminate one-word sentences. Do not ever use a big word when a smaller one would suffice.

Verbs have to be in agreement with their subjects. In a research paper, do not start sentences with conjunctions or finish them with prepositions. When writing formally, it is advisable to never split an infinitive because someone will (wrongly) complain. Avoid clichés like a disease. Always shun irritating alliteration. Use language which is simple and straightforward. Put together a neat summary.

14. Arrangement of information: Each section of the main body should start with an opening sentence, and there should be a changeover at the end of the section. Give only valid and powerful arguments for your topic. You may also maintain your arguments with records.

15. Never start at the last minute: Always allow enough time for research work. Leaving everything to the last minute will degrade your paper and spoil your work.

16. Multitasking in research is not good: Doing several things at the same time is a bad habit in the case of research activity. Research is an area where everything has a particular time slot. Divide your research work into parts, and do a particular part in a particular time slot.

17. Never copy others' work: Never copy others' work and give it your name because if the evaluator has seen it anywhere, you will be in trouble. Take proper rest and food: No matter how many hours you spend on your research activity, if you are not taking care of your health, then all your efforts will have been in vain. For quality research, take proper rest and food.

18. Go to seminars: Attend seminars if the topic is relevant to your research area. Utilize all your resources.

19. Refresh your mind after intervals: Try to give your mind a rest by listening to soft music or sleeping in intervals. This will also improve your memory. Acquire colleagues: Always try to acquire colleagues. No matter how sharp you are, if you acquire colleagues, they can give you ideas which will be helpful to your research.



20. Think technically: Always think technically. If anything happens, search for its reasons, benefits, and demerits. Think and then print: When you go to print your paper, check that tables are not split, headings are not detached from their descriptions, and page sequence is maintained.

21. Adding unnecessary information: Do not add unnecessary information like "I have used MS Excel to draw graphs." Irrelevant and inappropriate material is superfluous. Foreign terminology and phrases are not apropos. One should never take a broad view. Analogy is like feathers on a snake. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Never oversimplify: When adding material to your research paper, never go for oversimplification; this will definitely irritate the evaluator. Be specific. Never use rhythmic redundancies. Contractions shouldn't be used in a research paper. Comparisons are as terrible as clichés. Give up ampersands, abbreviations, and so on. Remove commas that are not necessary. Parenthetical words should be between brackets or commas. Understatement is always the best way to put forward earth-shaking thoughts. Give a detailed literary review.

22. Report concluded results: Use concluded results. From raw data, filter the results, and then conclude your studies based on measurements and observations taken. An appropriate number of decimal places should be used. Parenthetical remarks are prohibited here. Proofread carefully at the final stage. At the end, give an outline to your arguments. Spot perspectives of further study of the subject. Justify your conclusion at the bottom sufficiently, which will probably include examples.

23. Upon conclusion: Once you have concluded your research, the next most important step is to present your findings. Presentation is extremely important as it is the definite medium through which your research is going to be in print for the rest of the crowd. Care should be taken to categorize your thoughts well and present them in a logical and neat manner. A good quality research paper format is essential because it serves to highlight your research paper and bring to light all necessary aspects of your research.

INFORMAL GUIDELINES OF RESEARCH PAPER WRITING

Key points to remember:

- Submit all work in its final form.
- Write your paper in the form which is presented in the guidelines using the template.
- Please note the criteria peer reviewers will use for grading the final paper.

Final points:

One purpose of organizing a research paper is to let people interpret your efforts selectively. The journal requires the following sections, submitted in the order listed, with each section starting on a new page:

The introduction: This will be compiled from reference matter and reflect the design processes or outline of basis that directed you to make a study. As you carry out the process of study, the method and process section will be constructed like that. The results segment will show related statistics in nearly sequential order and direct reviewers to similar intellectual paths throughout the data that you gathered to carry out your study.

The discussion section:

This will provide understanding of the data and projections as to the implications of the results. The use of good quality references throughout the paper will give the effort trustworthiness by representing an alertness to prior workings.

Writing a research paper is not an easy job, no matter how trouble-free the actual research or concept. Practice, excellent preparation, and controlled record-keeping are the only means to make straightforward progression.

General style:

Specific editorial column necessities for compliance of a manuscript will always take over from directions in these general guidelines.

To make a paper clear: Adhere to recommended page limits.



Mistakes to avoid:

- Insertion of a title at the foot of a page with subsequent text on the next page.
- Separating a table, chart, or figure—confine each to a single page.
- Submitting a manuscript with pages out of sequence.
- In every section of your document, use standard writing style, including articles ("a" and "the").
- Keep paying attention to the topic of the paper.
- Use paragraphs to split each significant point (excluding the abstract).
- Align the primary line of each section.
- Present your points in sound order.
- Use present tense to report well-accepted matters.
- Use past tense to describe specific results.
- Do not use familiar wording; don't address the reviewer directly. Don't use slang or superlatives.
- Avoid use of extra pictures—include only those figures essential to presenting results.

Title page:

Choose a revealing title. It should be short and include the name(s) and address(es) of all authors. It should not have acronyms or abbreviations or exceed two printed lines.

Abstract: This summary should be two hundred words or less. It should clearly and briefly explain the key findings reported in the manuscript and must have precise statistics. It should not have acronyms or abbreviations. It should be logical in itself. Do not cite references at this point.

An abstract is a brief, distinct paragraph summary of finished work or work in development. In a minute or less, a reviewer can be taught the foundation behind the study, common approaches to the problem, relevant results, and significant conclusions or new questions.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Use comprehensive sentences, and do not sacrifice readability for brevity; you can maintain it succinctly by phrasing sentences so that they provide more than a lone rationale. The author can at this moment go straight to shortening the outcome. Sum up the study with the subsequent elements in any summary. Try to limit the initial two items to no more than one line each.

Reason for writing the article—theory, overall issue, purpose.

- Fundamental goal.
- To-the-point depiction of the research.
- Consequences, including definite statistics—if the consequences are quantitative in nature, account for this; results of any numerical analysis should be reported. Significant conclusions or questions that emerge from the research.

Approach:

- Single section and succinct.
- An outline of the job done is always written in past tense.
- Concentrate on shortening results—limit background information to a verdict or two.
- Exact spelling, clarity of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else.

Introduction:

The introduction should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable of comprehending and calculating the purpose of your study without having to refer to other works. The basis for the study should be offered. Give the most important references, but avoid making a comprehensive appraisal of the topic. Describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will give no attention to your results. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here.



The following approach can create a valuable beginning:

- Explain the value (significance) of the study.
- Defend the model—why did you employ this particular system or method? What is its compensation? Remark upon its appropriateness from an abstract point of view as well as pointing out sensible reasons for using it.
- Present a justification. State your particular theory(-ies) or aim(s), and describe the logic that led you to choose them.
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Approach:

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Procedures (methods and materials):

This part is supposed to be the easiest to carve if you have good skills. A soundly written procedures segment allows a capable scientist to replicate your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order, but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt to give the least amount of information that would permit another capable scientist to replicate your outcome, but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section.

When a technique is used that has been well-described in another section, mention the specific item describing the way, but draw the basic principle while stating the situation. The purpose is to show all particular resources and broad procedures so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step-by-step report of the whole thing you did, nor is a methods section a set of orders.

Materials:

Materials may be reported in part of a section or else they may be recognized along with your measures.

Methods:

- Report the method and not the particulars of each process that engaged the same methodology.
- Describe the method entirely.
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures.
- Simplify—detail how procedures were completed, not how they were performed on a particular day.
- If well-known procedures were used, account for the procedure by name, possibly with a reference, and that's all.

Approach:

It is embarrassing to use vigorous voice when documenting methods without using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result, when writing up the methods, most authors use third person passive voice.

Use standard style in this and every other part of the paper—avoid familiar lists, and use full sentences.

What to keep away from:

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings—save it for the argument.
- Leave out information that is immaterial to a third party.



Results:

The principle of a results segment is to present and demonstrate your conclusion. Create this part as entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Use statistics and tables, if suitable, to present consequences most efficiently.

You must clearly differentiate material which would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matters should not be submitted at all except if requested by the instructor.

Content:

- Sum up your conclusions in text and demonstrate them, if suitable, with figures and tables.
- In the manuscript, explain each of your consequences, and point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation of an exacting study.
- Explain results of control experiments and give remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or manuscript.

What to stay away from:

- Do not discuss or infer your outcome, report surrounding information, or try to explain anything.
- Do not include raw data or intermediate calculations in a research manuscript.
- Do not present similar data more than once.
- A manuscript should complement any figures or tables, not duplicate information.
- Never confuse figures with tables—there is a difference.

Approach:

As always, use past tense when you submit your results, and put the whole thing in a reasonable order.

Put figures and tables, appropriately numbered, in order at the end of the report.

If you desire, you may place your figures and tables properly within the text of your results section.

Figures and tables:

If you put figures and tables at the end of some details, make certain that they are visibly distinguished from any attached appendix materials, such as raw facts. Whatever the position, each table must be titled, numbered one after the other, and include a heading. All figures and tables must be divided from the text.

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Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implications of the study. The purpose here is to offer an understanding of your results and support all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of results should be fully described.

Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact, you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved the prospect, and let it drop at that. Make a decision as to whether each premise is supported or discarded or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."



Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work.

- You may propose future guidelines, such as how an experiment might be personalized to accomplish a new idea.
- Give details of all of your remarks as much as possible, focusing on mechanisms.
- Make a decision as to whether the tentative design sufficiently addressed the theory and whether or not it was correctly restricted. Try to present substitute explanations if they are sensible alternatives.
- One piece of research will not counter an overall question, so maintain the large picture in mind. Where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

When you refer to information, differentiate data generated by your own studies from other available information. Present work done by specific persons (including you) in past tense.

Describe generally acknowledged facts and main beliefs in present tense.

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<i>Introduction</i>	Containing all background details with clear goal and appropriate details, flow specification, no grammar and spelling mistake, well organized sentence and paragraph, reference cited	Unclear and confusing data, appropriate format, grammar and spelling errors with unorganized matter	Out of place depth and content, hazy format
<i>Methods and Procedures</i>	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
<i>Result</i>	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
<i>Discussion</i>	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
<i>References</i>	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring



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