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THE EFFECTIVENESS OF INTRAUTERINE BLOOD TRANSFUSION IN TREATING HDFN CAUSED BY RED BLOOD CELL ALLOIMMUNIZATION

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1. ABSTRACT

Background: Hemolytic disease of fetus and newborn (HDFN) due to maternal red cell alloimmunization showed a wide propagation worldwide, causing the increase in rates of mortality and morbidity. Intrauterine transfusion (IUT) is one of several treatments used to treat fetuses with HDFN disease by providing the baby with compatible blood, therefore increases the chance of survival. This research aimed to measure the effectiveness of IUT in relation to the survival chance of affected fetuses and the presence of hydrops by systematically reviewing published articles. **Literature review:** The way of performing intrauterine transfusion evolved over the years. Initially, blood was transfused into the peritoneum space and currently the blood components are transfused inside the umbilical cord in which absorbed into the fetal circulation. During the past years, a diversity of indications has been qualified, the main indication was RBC alloimmunization. In a retrospective study performed between 1999 and 2013, 87% of all neonates were having hemolytic anemia due to maternal alloimmunization. Pregnancies with HDFN caused by red cell alloimmunization are yearly having an increase in the number of affected fetuses that trigger the involvement of intrauterine blood transfusion. **Method:** To ensure the validity and reliability of the research, a sound methodology has been established that involved usage of the following measures, authentic sites like PubMed, official journals and most recent articles. The search strategy was based on the usage of keywords and synonyms reflecting the PICO elements of the research topic. **Result and**

discussion: A dramatic enhancement in prognosis of HDFN and neonatal outcomes was accomplished with availability and introduction of IUT. From the various studies included in this research, the Survival chance increased significantly and ranged between 81% and 100%. Currently in experienced hands, procedures related complications are as low as 1.2 % per procedure and loss rate of 0% reported in Pasman et al study. Nevertheless, prevention of fetal loss can be technically approached in cases that required early intravascular IUT, and for that systems need to be established for early referral diagnosis and antibodies detection to prevent or minimize the level of hydrops. **Conclusion:** Intrauterine blood transfusion is considered safe and effective technique in treating hemolytic disease of fetus and newborn caused by red cell alloimmunization, resulting in improved neonatal outcomes as it associates with lower rates of procedure related complications.

2. INTRODUCTION

2.1. Background

Hemolytic disease of fetus and newborn (HDFN) is a destruction of the red cells in the fetus and neonate by antibodies produced by the mother which can cause mild complications and severe problems reaching to death of the fetus. Pregnancies complicated with HDFN is one of the most critical health issues. Red cell alloimmunization is considered to be a major transfusion problem and providing blood to fetus may trigger the immune system. Worldwide, there is an increase in the rate of mortality and morbidity. A study done at SQU hospital revealed that the prevalence of alloimmunization caused by RhD showed up in 91 patients (7.3%) out of 1251(Aldughaishi T et al, 2016). These results were comparable to other Asian countries and the reasons proposed are close cultural and commerce relations. Fifty years back, HDFN was one of the most widely recognized reasons of perinatal mortality. Early delivery and neonatal exchange transfusions were the only treatment choices, until in the 1960s; intrauterine transfusion became available (Adamsons, K. et al. 1965). Intrauterine transfusion is a technique used to provide blood through the umbilical cord to an Rh-positive fetus. This technique tends to keep fetus in a healthy status until he or she grows enough to be delivered. The mortality rate of fetus increased dramatically because of severe anemia and hydrops but in order to prolong the life of the fetus, intrauterine transfusion is recommended although there are some potential risks. Therefore, it is essential to evaluate the efficiency of IUT in relation to mortality rate and the presence of hydrops fetalis by systematically reviewing published articles.

2.2. LITERATURE REVIEW

2.2.1. History: techniques used over years

In 1960s, a scientist called Liley introduced the first version of the series approaches for treating HDFN caused by red cell alloimmunization (Liley AW, 1963). Liely demonstrated a direct relationship between bilirubin concentration and the extend of fetal anemia. This rule considered to be a diagnostic element for fetal isoimmune anemia. During his experiment he intended to collect the amniotic fluid for bilirubin estimation but mistakenly he collected ascites and hence the idea of intraperitoneal transfusion clicked his mind as an efficient diagnostic technique to treat hemolytic diseases of fetuses. This technique carried out with the aid of x-ray to visualize the fetus position. Then a local anesthesia is applied, and a needle is inserted into the fetal peritoneum. In spite of the fact that peritoneal transfusion improves the rate of survival chance of fetuses, significant outcomes such as hydrops fetalis still poor (Liley AW, 1961). A new modality of intrauterine transfusion was established by Rodeck et al in 1981. In this technique, blood is transfused to the umbilical cord with the help of needle and guided by fetoscopy (Rodeck Ch et al, 1984). High accurate results perceived by ultrasound guidance encourage some scientists as Ferdinand Daffos and Jens Bang to conduct cordocentesis by injecting red blood cells directly into the fetal umbilical vein (Bang J et al, 1982). In 1990, Nicolini et al. devised the performance of intrauterine transfusion for intrahepatic part of the umbilical vein (Nicolini U et al, 1990), and this technique found to be more effective than the techniques that target the umbilical cord especially in incident posterior placenta (Van KIL et al, 2005). The period from 1987 and onwards, intrauterine transfusion evaluated to be the best method for treating HDFN and associated complications (Van KIL et al, 2004). However, many comorbidities can occur and even lethal ones.

2.2.2. Indication for intrauterine transfusion (IUT)

Fetal anemia can occur because of several reasons, hemolysis of normal red blood cell is one of them, and the rests are, RBCs produced are less than the normal level, dilution of erythrocytes, hemoglobin diseases and enzymatic disorders. Intrauterine transfusion should be kept in mind when it comes to hemolytic diseases of fetus and newborn (HDFN) as it has great outcomes, but to maximize the benefits of this procedure, a quite knowledge and being familiar with indication of IUT are very crucial. Red blood cell alloimmunization is considered to be the main indicator for IUT, however, there are other indicators that involved in this. For instance, human parvovirus B19 infection, fetomaternal hemorrhage, twin twin transfusion syndrome and placental tumors.

Bilirubin of amniotic fluid was initially assessed by Liley in 1961, amniocentesis process used to be the norm method for managing the red cell alloimmunization in pregnant ladies at that time. Amniocentesis done at week 18 to 20 on mothers with history of severely affected fetus or early fetal death, the reason behind doing this procedure is to know whether that particular women needs a frequent follow up or intervention in order to prevent the worse complications that could happen to her baby or even might result in fetus's death. This process done by testing the bilirubin level in amniotic fluid by taking the absorbance at 450 nm wavelength and plotting on a graph in which the Y= absorbance and X= gestational age in week or trimester, that graph called "Liley graph". In general, Liley graph has three zones for interpretation, the first zone indicates that the fetus is not affected or mildly harmed, the second zone emerges that the fetus is moderately affected and hence required close intervention, the third zone illustrates that the fetus is severely affected and therefore needs an immediate intervention. Anyhow, amniocentesis is an invasive method to do, so the scientists decided to find another way which does the same function. Doppler scan of the middle cerebral artery peak systolic velocity (MCA-PSV) considered to be non-invasive method, it can be done at 16-18 weeks of pregnancy period.

2.2.3. Red cell alloimmunization (RBC isoimmunization)

As name implies it is an immune response to alloantigen. Maternal RBC immunoglobulins can cross the placenta and reach the fetal circulation with a potential of causing hemolysis. In the past years, several specialized centers conducted IUT due to red blood cell alloimmunization with a sample size of 30-491 and illustrated a great survival rate that ranged from 80.5-93.5% (Van KIL et al, 2005) (Yinon Y et al, 2010) (Tiblad E et al, 2011) (Johnstone AC et al, 2012) (Osanan GC et al, 2012) (Lindenburg I et al, 2013). With the wide use of IUT as an advance technique along with the professional assessment more hydropic fetuses seemed to survive much better. Even so, long term morbidity may present on those fetuses. For instance, small studies conducted, yielded the presence of adverse outcomes after the delivery with a range of 2.8-13% (Farrant B et al, 2001) (Weisz B et al, 2009). Few years back in 2012 a large study performed to analyze the outcomes of intrauterine transfusion and extended for a period of 20 years (Lindenburg IT et al, 2012). The sample size was 451 fetuses with a total number of 1284 IUTs. Alloimmunization was caused by RhD, Kell, and Rhc with a percentage of 80%, 12%, 5% respectively. Fetuses with hydrops fetalis were 26% at the initial transfusion. At the first transfusion, the mean of gestational age was twenty-six weeks and the mean frequency of transfusion was three. The majority number of fetuses

(more than 95%) were intact in term of neurodevelopment which can be triggered by the presence of hydrops fetalis. Fetal hydrops can be prevented or minimized to the minimum level by early detection and the proper treatment intervention. The first version of intrauterine transfusion reported by Liley depends on the process of transfusing blood to the peritoneum space. The lymphatic system plays an important role as the RBCs are able to reach the fetal circulation through it. In case of severe fetal hydrops, this process of absorbing RBCs by lymphatic system will be inhibited. Although, intraperitoneal transfusion considered a suitable alternative intervention if IUT failed to be performed in the umbilical vein of the intrahepatic section. Instead, administration of maternal intravenous immunoglobulin (IVIG) with or without plasmapheresis in extremely affected pregnancies can postpone the first IUT and hence reducing procedure related complications (Fox C et al, 2008). The case reported in this study support that idea as six women who had a history of very severe anemia prior twenty weeks of pregnancy. All of them received IUTs in the period between 16-21 weeks of pregnancy with intravenous immunoglobulin. Out of the seven fetuses (one woman delivered twin), six fetuses survived.

2.2.4. IUT and associated risks

The types of risks divided into two categories, acute and long-term complications. Acute related threats occur after intrauterine transfusion intervention, an example for that is fetal distress which can happen during or after the procedure and known to be the most serious problem as it can result in fetus death or delivering the fetus earlier with potentials of prematurity or neonatal asphyxia (Van KIL et al, 2005) (Yinon Y et al, 2010) (Lindenburg I et al, 2013). Long related threats appeared in the fetus after a period of time, for instance, treating the neonate with IUT needs a lot of blood cell transfusion during the six months of life which can result in funnel of fetus erythropoiesis (Rath ME et al, 2010).

2.2.5. Improving outcomes of IUT

There are several ways that used to improve the outcomes of this intervention. Firstly, IUT with ultrasound guided procedure is essential to navigate the way and monitor the fetal status. Secondly, fetal paralysis is important to prevent the unexpected fetal motion and the detachment of the needle. Thus, fetal distress secondary to cord damage and artery puncturing can be avoided (Van KIL et al, 2005). Early detection and intervention can prevent or reduces hydrops. In addition, transplacental transfusion should be avoided and if necessary, carefully match IUT donors for immunogenicity triggers. Alternatively, one donor

used in a serial pattern for each fetus can prevent the recruitment of more antibodies during the IUT intervention (Rath ME et al, 2010).

2.2.6. Aims and objectives

The aim of this research is to estimate the efficacy of IUT and to know whether it is the best current treatment for hemolytic disease of fetus and newborn due to red cell alloimmunization or not. This objective is fulfilled by obtaining more precise information about IUT indications and associated risks and the way to overcome these risks. The outcomes that this research focused on included the survival rate (mortality rate), procedure related complications, hemoglobin levels, and the presence of hydrops among fetuses by systematically reviewing published articles.

3. REVIEW METHODS

Hemolytic disease of fetus and newborn (HDFN) is known to be a major cause of stillbirth. It causes the raise in morbidity and mortality rate in all over the world. To fight against this type of disease it comes essential to develop a safe intervention to prevent HDFN and generally improves the outcomes. IUT could be one of those safe interventions, but it needs to be evaluated in terms of efficiency and this research is conducted to complete the proposed aim. As health professionals, it's important to provide the maximum allocation of health care resources and improve medical services.

In order to ensure the validity of mentioned facts and evidences, a systematic search had been conducted in authentic sites like, PubMed, WHO library Database, life science journals, Medline, WebMD journal etc. A series of steps had been followed during a fixed time to reach a high quality articles which support this project with required information. The topic of this research focuses on the employment of intrauterine transfusion for managing hemolytic disease of fetus and newborn caused by red cells alloimmunization. The first step was making an evident strategy to gather articles with specific criteria to follow and avoid random searching while investigating articles or information related to our research topic. According to most researchers' methodologies, firstly we started with an essential step which is defining the topic scientifically to make it vibrant to readers. It was defined by using of dictionaries, encyclopedias, asking our research supervisor, and our personal words to find suitable terminologies that reflect the research topic elements evidently. To be more accurate and precise in searching, there are alternatives key words used. For instance, intrauterine transfusion, IUT, hemolytic disease of fetus and newborn, HDFN, red cells alloimmunization,

and red blood cells isoimmunizations, hydrops fetalis. Selecting these words make the method of collecting data more straightforward. In fact, synonyms and compound words were advantageous to get more detailed and extensive input. The main source that was used to collect data in short time and less effort is Google searching engine due to the huge amount of data uploaded there, and the easy accessibility to many authorities. During the search process we encountered an issue in accessing articles that require money, however; MOH e-library supplies variety of authentic journals for scientific researches that could be used by students or researchers. After searching for large numbers of articles from different sources we followed the filtration method which requires to narrow down the search methodology and make our target to specific articles. After achieving this step, we have read the articles in deep and understood each one to start analyzing data and writing proposal sections. In addition, inclusion and exclusion criteria depend on the study objectives. Each study has its own criteria that relies on desirable outcomes. All subjects with living babies, presence of hydrops fetalis, hemoglobin levels, survival chance, procedure related complications, and mortality rate were the inclusion criteria of the research. On the other hand, the exclusion criteria were operator's procedure details and dead born babies. The reason behind not involving the technical details of the procedure is due to the lack of information and the concealment of information in retrospective studies. Our research delimitations were focused on articles that are recent, published less than seven years back, and written in English language only. Lastly, the ethical considerations had been taken into account because any research study involved human participation should be done based on ethical standards and in accordance to Helsinki declaration of 1964.

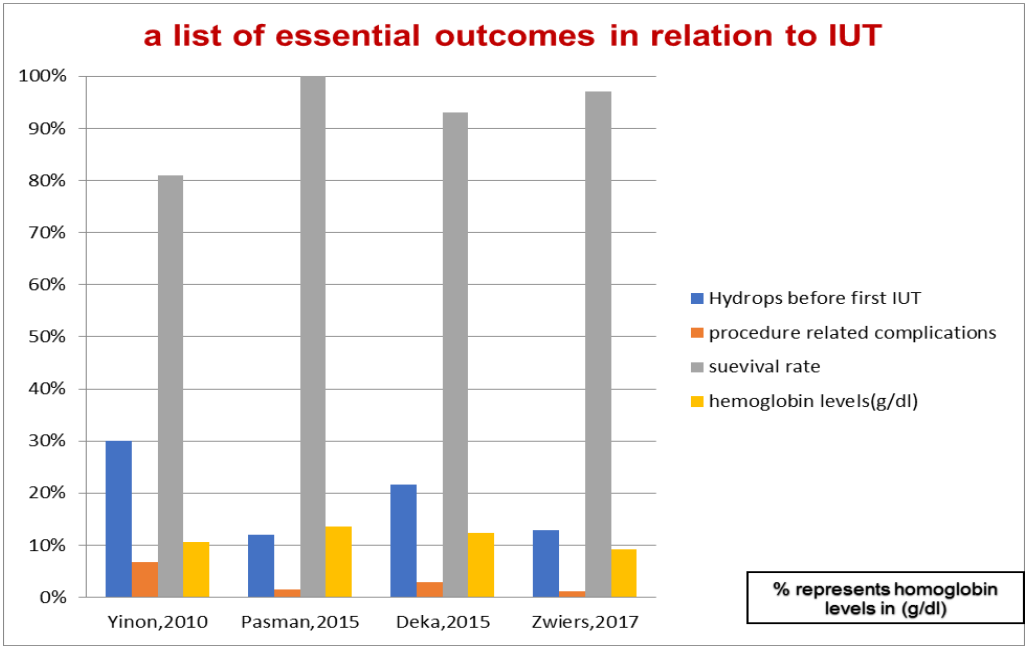
4. RESULTS

Table 1: Overall survival rates and other essential outcomes in relation to IUT.

Author, year	Sample size/ number of IUTs	Hydrops (%) before first IUT		Hemoglobin levels (g/dl) before and (after) first IUT		Procedure related complications		Loss rate		Survival rate	
Yinon, 2010	30/137	30%		3.7(10.6) median		6.7%		6 case		81%	
Pasman, 2015	56/135	12%		8.3(13.6) mean		1.5%		0 case		100%	
Deka, 2015	102/303	21.6%		5.67+/-2.6(NA) mean		2.96%		7 case		93%	
Zwiers, 2017	595/1685	P 1	P 2	P 1	P 2	P 1	P 2	P 1	P 2	P 1	P 2
		38%	12.9%	4.3(11.2) median	6.3(9.2) median	3.4%	1.2%	29 case	10 case	88.6%	97%

All the results illustrated in table 1 were copied exactly with the same values' units, P1 means phase one and P2 means phase two, as the study done by Zwiers was divided into two phases because of the large sample size that lasted for long period of time. NA is stand for not available although the hemoglobin levels at birth were presented in the discussion.

Table 2: Easy comparative visualization for several parameters.



All hemoglobin values were after the first IUT, with the exception of Deka et al study as it illustrated the value at birth.

5. DISCUSSION

5.1. Main findings and interpretation

Intrauterine blood transfusion is regarded as a safe procedure in treating hemolytic disease of fetus and newborn due to red cell alloimmunization, resulting in a better fetal outcome. The conclusive idea of the reported literature is supported by including four large and recent studies to gather an accurate information and set the rationale.

Deka et al (2015) shared their experience with intravascular IUTs performed in a period of four years. 102 cases were included that nominate it to be the largest study from a developing country (Deka et al, 2015). A survival chance of 93% was reported from fetuses subjected to intrauterine transfusions, that is comparable to other studies, Van Kamp et al (2005) and Tiblad et al (2011) of 86%, 91.3% respectively (Deka et al, 2015). There was no statistical difference between hydropic and non-hydropic fetuses in terms of survival chance in Deka D

et al series. 22 hydropic fetuses showed a survival rate of 91%. A lower survival rates were reported by Van Kamp et al, 78% in 80 hydropic fetuses and this indicates a significant difference while compared to Deka D et al research. This gap in the rates of survival between the two studies is probably explained by the remarkable improvement in ultrasonography and enhanced neonatal care (Deka et al, 2015). Hemoglobin levels before the first IUT and at birth were 5.67+/-2.6 g/dl, 12.32+/-3.0 g/dl respectively.

In a large study lasted for 27 years, Zwiers et al (2017) performed 1685 IUTs for 595 fetuses of 497 pregnant women. The study was divided into two phases, 1988-2000 with a total number of performed IUTs of 741, and 2001-2015 were the pregnant women received 937 IUTs. A comparable survival rate to many other studies was seen in the first phase (1988-2000) in which it was 88.6% and approached statistical significance in the second phase (2001-2015) where it was 97% (Zwiers et al, 2017). The presence of hydrops at first IUT was 38% in the first phase and 12.9% in the second phase of the study. hemoglobin levels at first IUT were 4.8 g/dl, 6.3 g/dl in the period of 1988-2000, 2001-2015 respectively. A noticeable improvement in both levels of hydrops and hemoglobin were observed after IUT treatment. The increase in survival chance presented in this study is likely because of the low disease severity at referral stage, low hydrops, and increased hemoglobin levels at the first IUT which all contribute for a better survival rates (Zwiers et al, 2017). The improved outcomes reflect the excellent Dutch screening programs in detection and prevention of RBC antibodies. The study revealed that the most factor associated with great survival rates is large number of IUT performed per year (mean, 62 annually compared to 38/year by Pasman et al) (Zwiers et al, 2017) (Pasman et al, 2015). It is advocated that every health institution should maintain a good experience in all aspects of intrauterine transfusion and that is not achieved unless a sufficient number of transfusions is done per year (minimum of 10/year) (Zwiers et al, 2017).

In a study done by Pasman and his colleagues (2015), 135 intrauterine transfusions for alloimmune anemia were performed on 56 fetuses in a period of 14 years (2000 – 2014). There was no neonatal or perinatal death reported therefore the survival chance was 100% (Pasman et al, 2015). This study is one of the few cohort studies that described the IUTs for alloimmunization since the great cohort published by Van Kamp et al (2005). In Pasman study there was no perinatal death documented, whereas 12 fetuses out of 254 were died during the pregnancy in Van Kamp series (Pasman et al, 2015) (Van Kamp, 2005). The reason behind the difference between the two studies can be explained by the higher

percentage of hydropic fetus of 21% in Van Kamp et al series compared to12% in Pasman et al. noteworthy is that early detection of antibodies contributes to reduce the occurrence of hydrops (Pasman et al, 2015). The mean of hemoglobin level before the IUT was 8.3 g/dl and improved to 13.6 g/dl after IUT.

Early IUT before 22 weeks of gestational age is technically more challenging as it associated with high peripartum rate loss (Yinon et al, 2010). Yinon et al revealed in their study that there is no statistical significance difference regarding fetal survival in terms of gestational age, as the survival chance in their series was 78% in hydropic cases and 80% in non-hydropic fetuses which is in agreement with many other studies in later gestational age (Yinon et al, 2010). In spite of the fact that the survival chance was comparable to other studies in later period of the gestation, it still doubted to be approved since it published from one study and there is a lack of information in regard to that particular point. So, it need to be proven by multi specialized centers as most studies agreed that lesser survival rates are expected in the incident of early intrauterine blood transfusion. Hemoglobin levels before first IUT was 3.7 g/dl and reached 10.6 g/dl after the first IUT. Hydrops fetalis presence was 30% before the initial IUT (Yinon et al, 2010).

However, being an invasive technique IUT has several procedure related complications(PR). Anywise, all mentioned studies illustrate commensurate data that has no significant impact on overall survival chance. The results of PR complications and (loss rates) were 2.1%(35 cases), 2.9%(7 cases), 1.5%(0 cases), 6.7%(6 cases) in Zwiers et al, Deka et al, Pasman et al, Yinon et al respectively (Zwiers et al, 2017) (Deka et al, 2015) (Pasman et al, 2015) (Yinon et al, 2010). There was considerable diversity in the type of procedure related complications and mortality (loss rates) among the four studies. In Zwiers et al, complications were preterm premature rupture of membranes (PPROM), intrauterine infections like E.coli, emergency cesarean section, and intrauterine death (IUD). Yinon et al reported some complications similar to Zwiers and added fetal bradycardia, hemorrhage in the cerebellum, and cerebellar hypoplasia. Pasman et al illustrated two main adverse events, prolonged post procedure hemorrhage and uterine contractions. Whereas in Deka study, some patients were afflicted with chorioamnionitis and other cases were admitted to intensive care unit due to severe birth asphyxia.

Sometimes the fetal loss is not related to the procedure but may due to underlying pathology, as in harshly anemic and hydropic patients. From different literature, fetal loss is associated

with various factors including, fetal hydrops, severity of fetal anemia, early gestational age, avoiding the use of fetal paralysis, and experience of the operator (Van Kamp et al, 2001) (Van Kamp et al, 2005) (Dadhwal et al, 2010) (Lindenburg et al, 2011) (Tiblad et al, 2011) (Ayliffe-Johnstone et al, 2012) (Osanan et al, 2012) (Lindenburg et al, 2013).

5.2. Strengths and limitations

One of the strengths of this research is that all articles utilized in here have been critically appraised by CASP tool to ensure the validity of the data retrieval. Although the retrospective studies are potentially allowed for selection and other type of biases, the selection of all articles included for the literature was done randomly to reduce the biases and the search of articles was totally dependent on a pre-planned searching strategy and not to meet our outcomes of interest. In addition, very large and recent studies from authentic journals were used to discuss and analyze the aims and objectives of the research. Fortunately, the random selection of the studies to be included, gathered series from different countries which perfectly represent the target population. And since the conducted research is collected from different populations, it can be suitable for generalization and implementation for professional fields.

There are many limitations that should be considered for analysis and interpretation of the result. Firstly, there was an accessing difficulty as some journals required money and specific registration and that obstacle partially solved by contacting MOH e-library. Some articles were in languages rather than English. Noteworthy is that the technical details of procedures performed by operators cannot be gathered unless it mentioned by the authors and that is difficult to get in retrospective study.

5.3. Further research and recommendations

This research focused on the effectiveness of IUT in treating HDFN caused by red cell alloimmunization. However, there are several important gaps remaining in the literature that should be addressed scientifically. Further study is required to compare statistically between IUT and non-invasive techniques. In addition, more studies need to be established in early IUT management and search for advanced therapies like stem cell and gene therapy. Noteworthy, more studies need to be published about the relationship between early intrauterine transfusion and gestational age. Since the fetal paralysis proven to be associated with lower procedure related complication it is advocated for routine use.

6. CONCLUSION

To sum up, in experienced hands, IUT is a safe and effective technique in treating HDFN due to RBC alloimmunization. It results in great neonatal outcomes as it associates with minimal percentage of loss rates and procedure related complications.

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Appendix 1:

Search strategy

Data base	Key words	No of articles found	Articles selected
NCBI (linked to PubMed)	IUT, HDFN, red cell alloimmunization	>10 articles	Randomly one
NCBI (linked to PubMed)	IUT AND red cell alloimmunization	>10 articles	Randomly one
NCBI (linked to PubMed)	IUT related complications AND red cell alloimmunization	>10 articles	Randomly one
NCBI (linked to PubMed)	Early IUT AND red cell alloimmunization	1 article	one

Appendix 2

Evidence table

Title of article	Authors/Year Of publication	Type OF study	Summary (relevant to the research)
Intrauterine transfusion for fetal anemia due to red cell alloimmunization: 14 years experience Leuven	Pasman SA et al (2015)	Retrospective cohort study	- The four elected articles showed high relevance to our research aims and objectives. - In all articles, our focus was pointed in accordance to the proposed inclusion criteria (All subjects with living babies,). - All evidences are critically appraised using CASP tool for the corresponding study type.
Complications of intrauterine intravascular blood transfusion: lessons after 1678 procedures	Zwiers C et al (2017)	Retrospective cohort study	
Early intrauterine transfusion in severe red blood cell alloimmunization	Yinon Y et al (2010)	Retrospective cohort study	
Perinatal survival and procedure related complications after intrauterine transfusion for red cell alloimmunization.	Deka et al (2015)	Prospective cohort study	