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EARLY USE OF VAGINAL PROGESTERONE AND CERVICAL PESSARY TO REDUCE PRETERM BIRTH RATES IN TWINS

A Thesis Presented to
The Faculty of the School of Medicine
Yale University

In Candidacy for the degree of
Master of Medical Science

July 30th, 2021

X_____

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Abstract

Preterm birth is one of the most common causes of neonatal morbidity and mortality. The incidence of preterm birth can be reduced by treatment with vaginal progesterone or cervical pessary. However, no studies have supported a treatment regimen that can reduce preterm birth in twin pregnancies**. In this study, we will examine whether a combination therapy of vaginal progesterone and cervical pessary can reduce preterm birth in women with dichorionic-diamniotic twin gestations.** We will enroll pregnant women with dichorionic-diamniotic twins at 11-14 weeks' gestation from a local hospital in a randomized control trial. This novel approach may help establish a new management option for twin pregnancies and significantly decrease the morbidity and mortality that affects them.

CHAPTER 1: INTRODUCTION

1.1 Background

1.1.1 Preterm birth epidemiology in the United States

Preterm birth is defined as delivery prior to 37 completed weeks of gestation and is one of the most common complications of pregnancy.[1,2] In the United States, preterm birth is the most common cause of neonatal morbidity and mortality accounting for at least one-third of infant deaths and is the second most common cause of death in children under 5 years of age.[3,4] Preterm neonates that are born before 32 weeks of gestation are at a higher risk of complications, such as respiratory distress syndrome (RDS), intra ventricular hemorrhage (IVH), and necrotizing enterocolitis (NEC).[4,5] Premature newborns may require prolonged hospitalization and lifelong medical treatments.[4,6] While various etiologies account for preterm delivery, one of the major risk factors for preterm delivery is multiple gestations (i.e. twins and higher order multiples).

The rate of twin births has risen 76% from 1980 to 2009, and the rate of preterm delivery among multiple gestations has also increased during this time period.[8] Studies have shown that there is a causal effect between twin pregnancies and preterm birth.[7,8] Other studies have also discovered that the rate of preterm births due to twin pregnancies has increased from 11.8% in 1995 to 16.7% in 2013.[7] In the United States, twins account for 10% of premature births and about 23% of all preterm births that occur before 32 weeks of gestation.[9] Twins can suffer from both spontaneous and indicated preterm births, but more than 70% of preterm births in multiple gestations are spontaneous.[13] Increased incidence of twin gestations combined with their increased preterm birth in twin gestations is one of the major factors for the persistently high rates of preterm birth overall in the United States.[7]

Twins born prematurely have a 4 times higher risk of mortality when compared to a singleton born at the same gestational age.[10] Similarly, late preterm birth (gestational age between 34 weeks 0 day and 36 weeks 6 days) has a minimal effect on increasing mortality in singletons. However, late preterm births in twins lead to 3 times higher risk of mortality.[7] Overall, premature birth is responsible for greater than 50% of all neonatal deaths for multiple gestations.[11] Although a great deal of effort has been placed into reducing the incidence of preterm births, the majority of these efforts are being focused on singleton pregnancies.[7-11] As a result, the effectiveness of interventions to reduce preterm births in twin pregnancies is not well understood.[7-10] Given that this is a high-risk population, a greater emphasis needs to be placed on establishing effective interventions to reduce the rate of preterm births in twin gestations.

1.1.2 Screening for Preterm Births

Accurately predicting preterm delivery has been challenging in patients without a history of prior preterm delivery. Currently, transvaginal ultrasound (TVU) is used to measure cervical length to stratify patients for risk of cervical insufficiency and preterm delivery.[12] Screening asymptomatic singleton pregnancies with TVU is usually done between 16 and 23 weeks' gestation.[12,19] In singleton gestations, a cervical length of less than 35mm is considered short, and if it is less than or equal to 20mm, vaginal progesterone treatment is indicated.[12,19] Asymptomatic multiple gestation TVU screening is not recommended in clinical practice, despite carrying a higher risk than singleton gestations, because there are no effective treatment modalities developed for multiple gestations yet.[12] Even with screening, predicting premature birth can be very difficult in multiple gestations as the association between

cervical length and risk of preterm labor is not as well understood in these cases.[12,19] Since spontaneous preterm births are much more common than indicated preterm births in twin pregnancies, observing and waiting for the cervical length to change could delay proper treatment.

1.1.3 Current Treatment Options for Reducing Preterm Deliveries in Multiple Gestations

Currently, limited treatment options are available to patients with multiple gestations that are at a high risk of delivering prematurely. Studies have shown that progesterone supplementation significantly decreases the rate of preterm birth in high-risk singleton pregnancies.[7] During the past several decades, multiple studies have attempted to discover effective treatments that could lower the incidence of premature birth in twin pregnancies and delay birth to increase the gestational age.[13-16] Treatment modalities, such as intramuscular 17 alpha-hydroxyprogesterone or natural vaginal progesterone, that were effective in preventing premature birth in singleton pregnancies, did not show the same benefit in twin pregnancies.[15,16] Additionally, utilizing higher dose vaginal progesterone did not demonstrate any benefit.[17] While progesterone may not be effective at preventing preterm birth in twins, combining it with a cervical pessary may produce a greater therapeutic response.[13-16]

Cervical pessary is a round silicone medical device placed at the opening of the cervix in women at risk of preterm birth, usually between 16 weeks and 22 weeks of gestation, has shown conflicting results.[18,19,31] Studies have shown it can reduce preterm birth before 34 weeks of gestation in singleton pregnancies with a short cervical length/cervical insufficiency.[19,20] However, there are no changes in neonatal morbidity or mortality between the two singleton groups.[19-21] Subsequent studies

could not duplicate these results and found no difference.[19-21] Studies that specifically utilized cervical pessary in unselected twin pregnancies found no overall treatment benefit in the pessary group, but the results showed that it significantly decreased premature birth rates before 32 weeks' gestation in a certain population of women with cervical insufficiency who had a cervical length was less than the 25th percentile (< 38mm).[19,24,] Furthermore, the rate of composite perinatal outcomes was significantly lower in this subgroup of women compared to the placebo group.[22-24]

Although the effects progesterone and cervical pessary have shown conflicting results across studies, it does reveal that they might have a potential as a treatment modality, especially for women with twin gestations and cervical insufficiency.[19,25,26] Because cervical shortening can be detrimental to twin pregnancies, resulting in preterm birth and severe neonatal morbidity and mortality, it might be beneficial to utilize a cervical pessary with progesterone since it has not been shown to cause any negative side effects.[18,19,24-26] Generally, a cervical pessary is inserted around 16 to 22 weeks' gestation for both singleton and twin pregnancies.[19] This new intervention might potentially improve outcomes by implementing it alongside daily vaginal progesterone between 11 to 14 weeks, which will be continued until the twins are delivered or until 38 weeks' gestation.[19]

1.1.4 The Role of Supplemental Progesterone on Reducing Preterm Birth Risk

Progesterone is a steroid hormone that has essential roles throughout pregnancy and is involved during the delivery process.[16,27] It is imperative in the process of uterine contractions, sustains decidualization, and has a role in promoting the immunity of the mother against fetal semi-allografts.[27] A number of studies have observed an increasing level of progesterone from conception to delivery.[17,28,29]

Progesterone hormone receptors are found throughout the uterus and cervix and regulate the effects of progesterone in pregnancy.[27] Progesterone's effects on the myometrium in the uterus allows for remodeling to facilitate the growth of the fetus.[27,28] Progesterone produced by the placenta, ovaries, and adrenal glands maintain uterine quiescence to prevent the onset of labor.[16,27,28]It inhibits the propagation of uterine contractions by preventing the synthesis of cell gap junctions in the myometrium.[27] Additionally, it acts to inhibit oxytocin and prostaglandin signals that are involved in the initiation of the parturition process.[27] The integrity of the cervix, which further facilitates gestation, is maintained through the actions of progesterone which prevents the breakdown of collagen in the cervical stroma.[27]

Low serum levels of progesterone have been correlated with an increased risk of preterm birth and threatened miscarriage.[19,27,29] Generally, when a woman has reached full term of gestation, a reduction in progesterone levels or modulation of progesterone receptors results in the onset of labor.[29] This is associated with an increase in uterine contractility, which is subsequently followed by the delivery of the fetus.[29] Thus, women that have a suboptimal level of progesterone production are at risk of early delivery.[19,29] As a primary preventative measure, there has been a particular focus on the role of supplemental progesterone on reducing the risk of preterm delivery. The route of administration has been shown to have immense effects on the efficacy and safety of its use in the prevention of preterm delivery.[19,27,29] It is been advised that oral progesterone should be avoided given that first-pass hepatic metabolism reduces its bioavailability, while simultaneously exposing the mother to an increased risk of side-effects.[16,27] Vaginal administration of progesterone bypasses first-pass hepatic metabolism and has local endometrial effects, is rapidly absorbed, has minimal systemic side effects, and a high bioavailability.[16] As a result, vaginal

administration is considered to be safe in pregnancy and has been approved by the United States Food and Drug Administration (FDA) in 2011.[16] A number of studies have shown that progesterone supplementation in pregnancy can have the same effects as endogenous progesterone and consequently facilitate fetal growth, maintain gestations, and prevent early onset labor by impeding uterine contractility, thus reducing the risk of preterm deliveries and consequent neonatal morbidity and mortality.[16,17,27-29] Although progesterone supplementation in pregnancy has been proven to have significant positive outcomes in singleton pregnancies, the effect of this intervention on multiple pregnancies is still not well understood.[16,17,27-29]

1.1.5 The Future of Interventions for Treatment of Preterm Births in Multiple Gestations

Novel and effective treatments are urgently needed to decrease the incidence of preterm birth, and subsequent neonatal morbidity and mortality, in twin gestations. A prospective open-label randomized controlled clinical trial is proposed to investigate the utility of late first trimester combined vaginal progesterone and cervical pessary use to prevent preterm delivery in unselected dichorionic-diamniotic (DCDA) twin gestations. This intervention differs from previous studies on twin preterm birth treatments because it implements cervical pessary alongside progesterone rather than investigating their use as monotherapies. Because this study consists of unselected twin gestations, cervical length would not be used for inclusion or exclusion study criteria. Using unselected twins allows for the intervention to start between 11 to 14 weeks' gestation rather than 16 to 22 weeks, in contrast to other studies.[19] Starting the intervention between 11 to 14 weeks' gestation could allow for a prophylactic treatment, which might increase the average gestational age in twin

pregnancies. Since a short cervical length is a major risk factor for premature birth, starting treatment earlier might prevent or delay the initial shortening process. Using a new method of implementing treatment sooner in the pregnancy might prove to increase gestational age.

With multiple studies being unable to find a solution to delaying preterm birth in twins, unexplored novel ideas must be tested to discover an effective treatment intervention for this ongoing issue. Intervention involving both vaginal progesterone and cervical pessary can easily be introduced earlier in pregnancy and used in clinical practice if proven to be effective, because they are non-invasive, affordable, have no known adverse effects, and could potentially lead to a decrease in the overall cost of care.[19,25]

1.2 Problem Statement

Even with the significant increase in risk, incidence, morbidity and mortality, there are no effective treatment options for preventing preterm birth in twin gestations.[5,6] Previous clinical trials that studied the effectiveness of interventions to prevent preterm birth in singleton gestations have been unable to provide statistically significant results for twin pregnancies.[5] Novel studies must be done to explore new ways to effectively use these interventions to treat premature birth in twin gestations, or definitively determine they do not work, prompting investigation into completely different treatments. In either case, further research is needed to improve the severe morbidity and mortality associated with preterm birth and twin pregnancies.

1.3 Goals and Objectives

The goal of this study is to investigate the effectiveness of combined vaginal progesterone supplementation and cervical pessary initiated in the late first trimester in prolongation of gestation and prevention of preterm delivery in unselected DCDA twin gestations. The primary objective of this study is to compare the effect of combined vaginal progesterone supplementation and cervical pessary (starting between 11 to 14 weeks' gestation until 37 weeks' gestation or birth) vs. no intervention in women pregnant with twins on the rate of preterm birth in DCDA twin gestations. Studies designed to decrease preterm birth has been done before and is an easy to track dichotomous outcome.

The secondary objective of this study is to investigate the effect of this intervention on a composite outcome of overall gestational age; level of preterm birth grouped by certain gestational ages (before 35, 34, 32 and 28 weeks of gestation); cervical length changes throughout pregnancy; birth weight; and neonatal morbidity and mortality in pregnancies treated with combined vaginal progesterone supplementation and concomitant cervical pessary initiated in the late first trimester in unselected DCDA twin gestations. Neonatal morbidity and mortality will be assessed by determining and comparing the APGAR score; admission to and time spent in the neonatal intensive care unit (NICU); and composite neonatal outcomes (the occurrence of any of the following events: RDS, IVH, sepsis, NEC, and death before hospital discharge) in the intervention group and the control group.

Comparing overall gestational age adds novelty to the study and sets it up for success. Because preterm birth is extremely common and difficult to treat in twin gestations, it is essential to conduct a study that can also establish a statistically significant treatment to lengthen average gestational age. Discovering statistically

significant improvements in these outcomes could also establish this intervention as the treatment of choice for preventing morbidity and mortality in premature twin gestations.

1.4 Research Questions

1. In twin gestations, without a maternal history of preterm birth, can the early (11-14 weeks' gestation) administration of both a daily vaginal progesterone and cervical pessary cause a reduction in preterm delivery rates compared to controls that undergo standard antenatal care?
2. In twin gestations, without a maternal history of preterm birth, can the early (11-14 weeks' gestation) administration of both daily vaginal progesterone and cervical pessary decrease composite neonatal morbidity and mortality compared to controls, who will receive the standard of care?

1.5 Hypothesis

It is hypothesized that the combined use of vaginal progesterone and cervical pessary from 11 to 14 weeks' gestation (until 37 weeks' gestation) will result in a 20% decrease in the rate of preterm births in DCDA twin gestations compared to women that receive standard cares during the same timeframe.

1.6 Definitions

1. **Vaginal progesterone:** Vaginal progesterone is a form of medical intervention that involves vaginally administering a tablet formulation containing either a natural or synthetic form of the endogenous steroid hormone progesterone.[30] Upon insertion, the progesterone binds to the progesterone receptor, which

results in receptor phosphorylation, the dissociation of heat shock proteins, and transcription activation.[30] The activation of the progesterone receptor results in an increase in estrogen metabolism and a reduction in the number of viable estrogen receptors.[27,29,30] Progesterone results in a decrease in uterine contractility in pregnancy, secretory endometrial changes, and results in the maintenance of pregnancy.[27,30]

2. **Cervical pessary:** A cervical pessary is a round silicone medical device that is placed at the opening of the cervix. This medical device is placed early in pregnancy in women that are at risk of preterm birth and is removed later when this risk has elapsed.[31]
3. **Gestation:** The time period between conception and birth.[32]
4. **Preterm:** The birth of an alive neonate prior to completion of 37 weeks of gestation. Subcategories include moderate to late preterm (32 to 37 weeks), very preterm (28 to 32 weeks), and extremely preterm (less than 28 weeks).[33]

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CHAPTER 2: REVIEW OF LITERATURE

2.1 Introduction

The purpose of this literature review was to analyze the available evidence that is currently available in preterm birth management in twin gestations. Additionally, it aimed to identify which medical intervention was most effective at preventing preterm birth in twin pregnancies (vaginal progesterone administration vs. cervical pessary plus expectant management).

2.1.1 Excisional Sources

An a-priori designed protocol model was used to perform this review. An electronic search of PubMed, Scopus, Ovid, MEDLINE and Cochrane was conducted from July 2020 to July 2021 utilizing a combination of relevant keywords, medical subject heading terms, and word variants for “preterm birth” and “twin pregnancies”. The full list of keywords used in different combinations is outlined in table 1. Another hand search was performed in relevant articles to determine if their reference lists contained any additional relevant reports. The PRISMA guidelines were followed.

Table 1: The full list of keywords used to search databases for relevant literature

Keywords Used
Twin gestation
Multiple pregnancies
Multiple fetuses
Preterm birth
Gestational age
Cervical pessary
Vaginal progesterone
Tocolytic therapy
Cesarean section
Vaginal infections
Vaginal discharge
Steroid administration

Intra-uterine death
Neonatal death
Perinatal death
APGAR score
Birthweight
RDS
IVH
NEC
Sepsis
Retinopathy of prematurity
Bronchopulmonary dysplasia
Mechanical ventilation
NICU

2.1.2 Eligibility Criteria and Main Outcome Measures

The inclusion criteria for studies included randomized control trials in which twin gestations were randomized to either a control group (standard treatments or a placebo) or an intervention group receiving treatment for the prevention of preterm birth (any type of cervical cerclage, progesterone, cervical pessary, or a combination of these). The primary outcomes included preterm birth that was < 34 weeks of gestation. Secondary outcomes included: Preterm birth < 24 weeks; preterm birth < 28 weeks; preterm birth < 32 weeks; preterm birth < 37 weeks; gestational age at delivery (in weeks, continuous variable); intra-uterine death (either twin after 22 weeks of gestation); neonatal death (either twin up to 28 days of life); perinatal death (the sum of intra-uterine deaths and neonatal deaths); APGAR score <7 (calculated at 5 minutes); birthweight < 2500 g; RDS; NEC; sepsis; the need for mechanical ventilation; admission to the NICU. The primary and secondary outcomes were investigated in an unselected cohort of twin gestations.

2.1.3 Data Collection and Analysis

Relevant studies were extracted and reviewed by an independent third party to ensure the reliability and quality of included research. The risk of bias in the included studies was assessed through the Revised Cochrane risk-of-bias tool for randomized control trials. Each study was assessed for risk of bias arising from five domains: missing data, randomization procedures, protocol deviations, measurement, and reporting of results. The risk of bias was considered low if at least four of the domains were rated as being low risk, with at least one of these domains being random allocation to groups and allocation concealment. The strength of the recommendations and quality of the included evidence was assessed through the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) method.[4]

2.1.4 Results

Overall, 78 articles were identified, 64 were not included because they did not meet eligibility, 14 did. I especially focused on these 3 studies for the literature review after been deemed relevant to the current study (outlined in table 2). Other articles were also reviewed to look at confounders that might affect this proposed study.

Table 2: General characteristics of the included studies

Author	Year	Country	Period Considered	Inclusion Criteria	Intervention	Description of intervention
Merced et al.[1]	2019	Spain	2010 to 2014	Twin pregnancies, minimum maternal age 18 years, arrested preterm labor, cervical insufficiency, and gestational age between 24 and 33 weeks	Cervical pessary placement	65mm x 25 mm x 35 mm cervical pessary
Rehal et al.[2]	2021	England, Spain, Italy, Bulgaria, France, and Belgium	2017 to 2019	Maternal age older than 18 years, 2 live fetuses at the 11 to 13 weeks' scan, dichorionic or monochorionic diamniotic twin pregnancies, fluency in the local language	Vaginal progesterone	300mg bid from 11 to 14 until 34 weeks' gestation
Shabaan et al.[3]	2018	Egypt	2015 to 2017	Naturally conceived, uncomplicated pregnancy, dichorionic diamniotic twin gestation, no major fetal abnormalities	Vaginal progesterone	400mg administered once daily ("bedtime") through vaginal pessary

2.2 Effect of Vaginal Progesterone Administration and Cervical Pessary Insertion on Singleton Pregnancies

A review of the effect of vaginal progesterone administration and cervical pessary insertion on singleton pregnancies was conducted to determine the rate of reduction of preterm births that could be expected from using the same intervention in twin gestations. A cohort study conducted by Stricker et al. (2016) in 53 pregnant women with singleton gestations found that treatment with combined cervical pessary and progesterone did not result in a reduction in preterm deliveries compared to cervical pessary alone.[23] Delivery prior to 34 weeks of gestation occurred in 32.1% of participants that were given the combined intervention compared to 24.5% of participants that received monotherapy with cervical pessary.[23] However, it was found that there was a reduction in admission to the NICU in women that received combined treatment compared to monotherapy.[23] In contrast, a retrospective evaluation conducted by Tajima et al. (2020) on 95 pregnant women with singleton gestations

found that there was a 22.2% reduction in the rate of preterm deliveries in women that received combined vaginal progesterone and cervical pessary compared to women that received tocolysis (7.0% compared to 30.8% rate of deliveries before 36 weeks of gestation).[24] Similarly, Daskalakis et al. (2018) discovered through a prospective cohort study that combined vaginal progesterone and cervical pessary reduced the rate of preterm births in singleton pregnancies by 11.9% when compared to no intervention (44.4% vs. 32.5%).[25] Thus, it can be expected that if similar results are observed in the current study, combined vaginal progesterone and cervical pessary should have an estimated effect in reducing preterm birth rates in twin gestations by 10 to 20%.

2.3 Review of Empirical Literature on the Effect of Cervical Pessary Insertion on Preterm Birth Rates in Twin Gestations

To date, there has been no intervention that has been proven to be effective in reducing preterm birth rates in twin gestation, particularly in cases of known risk factors including a cervical insufficiency and threatened preterm labor. Merced et al. (2019) conducted an open, randomized, controlled trial to determine if cervical pessaries were beneficial in preventing birth before 34 weeks of gestation in women with twin pregnancies with the aforementioned risk factors.[1] Their results revealed that the use of cervical pessary vs. no intervention resulted in a significant reduction in birth rates before 34 weeks of gestation (16.4% vs. 32.3%).[1]

Similar findings were also seen in a few other studies. A retrospective cohort identified women with cervical insufficiency (cervical length < 20 mm) and assessed the association of cervical pessary inserted before 28 weeks of gestation in twin pregnancies compared to no intervention.[7] Findings were suggestive of lower incidence of delivery at < 32 weeks (p=0.05), prolonged interval to delivery (p=0.025), and reduced incidence of

severe neonatal morbidity (p=0.04).[7] Others studies showed that cervical pessary reduced preterm birth before 34 weeks' gestation in singleton pregnancies with a short cervical length.[5,8] However, neonatal morbidity did not differ between the two groups.[5,8,9]

Studies that specifically utilized cervical pessary in unselected twin pregnancies found no overall treatment effect in the pessary group, but the results showed that it significantly decreased premature birth rates before 32 weeks' gestation in a certain population of women whose cervical length was less than the 25th percentile (< 38mm).[5,10] Furthermore, the rate of composite perinatal outcomes was significantly lower in this subgroup of women compared to the placebo group.[10-12]

2.4 Review of Empirical Literature on the Effect of Vaginal Progesterone Administration on Preterm Birth Rates in Twin Gestations

Intramuscular/vaginal has been shown to be effective in reducing the rate of preterm birth in singleton pregnancies, with no additional benefit at higher doses.[13-16] A meta-analysis conducted in 1990 collated evidence from 7 controlled trials for the outcomes of progesterone agents during pregnancy.[17] Only women who were at high risk of preterm birth were selected and the outcomes also included neonatal morbidity and mortality. Overall, there was no statistical effect of progesterone agents during pregnancy with neonatal morbidity and mortality. However, intramuscular 17 alpha-hydroxyprogesterone caproates did decrease the overall incidence of preterm birth.[17] There has been a renewed interest in the outcomes of vaginal progesterone for recurrent or at-risk mothers for preterm birth. Two clinical trials demonstrated a reduction in preterm birth with both natural vaginal progesterone and synthetic progesterone administered intramuscularly.[18,19] However, considerations for the impact of prolonging

gestation on neonatal morbidities including respiratory distress are also of interest. Another clinical trial randomized women between 18-23 weeks with either live singleton or twin pregnancy with a previous history of preterm birth to administer vaginal progesterone. There were no major differences in the incidence of respiratory distress syndrome (10.5% in the intervention group vs. 10.6% in the placebo group) (95% CI 0.69-1.53, p=0.905).[20] There were also no differences in the rate of maternal morbidities associated with side effects (9.9% in the intervention group vs. 7.3% in the placebo group) (95% CI 0.85-2.15, p=0.204).[20] Overall, data has not been conflicting regarding neonatal outcomes yet there is improved gestational age when vaginal progesterone is administered at 18-23 weeks.[19,20]

Rehal et al. (2021) aimed to determine if the positive effects of vaginal progesterone could be extrapolated to multiple gestations. They conducted a randomized, placebo-controlled, double-blind trial to determine if the administration of daily vaginal progesterone 600mg from 11 to 14 weeks until 34 weeks' gestation in twin pregnancies influenced the rates of spontaneous preterm births. They observed a spontaneous births between 24 to 34 weeks of gestation among the intervention group of 10.4% in the progesterone group compared to 8.2% in the placebo group (95% CI 0.88-2.05, p=0.17).[2] However, they were able to find a minor benefit in preventing preterm birth prior to 32 weeks' gestation in those with a cervical length of < 30mm (p=0.08).[2] This indicates that progesterone may be beneficial in preventing preterm birth between 24 to 32 weeks of gestation, however, this beneficial effect might not extend to the third trimester of pregnancy.

Shabaan et al. (2018) performed a randomized, open-label, controlled trial on 70 pregnant women with twin pregnancies to determine the effect of vaginal progesterone administration on the rates of preterm delivery. They compared these

rates to women receiving no additional treatment beyond standard care. Unlike Merced et al. (2019), Shabaan et al. (2018) did not observe a statistically significant difference between preterm birth rates (delivery < 37 weeks) in women receiving vaginal progesterone and the control group (16.9% vs. 25.4%, p=0.06).[1,3] Furthermore, Shabaan et al. (2018) found that the mean gestational age between the two groups was similar.[3]

2.5 Effect of Vaginal Progesterone and/or Cervical Pessary on Preterm Birth Rates in Twin Gestation

A randomized controlled trial compared the efficacy of cervical pessaries with vaginal progesterone for twin pregnancies at 16-22 weeks among women who had a cervical length < 38 mm.[21] They observed a 16% (n=24) preterm birth rate < 34 weeks' gestation in the cervical pessary group compared to 22% (n=33) in the progesterone group.[21] Those with cervical insufficiency who had a cervical length of < 29 mm, pessary reduced the birth rate before 34 weeks from 46% (n=16) to 21% (n=10) with improvement in perinatal outcomes.[21] Although progesterone and cervical pessary have shown conflicting results, these studies reveal that they also show promising ones, especially for women with twin gestations and cervical insufficiency.[5,22]

The combined use of vaginal progesterone and cervical pessary has also been observed in twin pregnancies to determine the outcome of preterm births at 18-27 weeks of gestation.[26] Among women with cervical insufficiency (cervical length of < 26 mm), there were no statistical differences between intervention and standard of care groups for preterm birth < 34 weeks (40.0% in the intervention group vs. 18.8% in the standard of care group, p=0.07).[26] In terms of the birthweight, there were also no statistical differences between intervention and standard of care groups.[26]

However, an important predictor of preterm birth (< 34 weeks) in ongoing pregnancy for women receiving both vaginal progesterone and cervical pessary in mothers with twin pregnancies was the previous history of preterm birth (< 37 weeks) (p=0.031).[26] A recent meta-analysis reviewed the outcomes of vaginal progesterone, cervical pessary and cervical cerclage among women with twin pregnancies and short cervical length (< 26 mm) with no risk in reduction of preterm birth < 34 weeks.[27]

2.6 Review of Empirical Literature on the Effect of Vaginal Progesterone and/or Cervical Pessary on the Rates of Neonatal Morbidity and Mortality

In addition to reducing preterm birth, an important factor to measure is whether the intervention decreases neonatal morbidity and mortality. Although Merced et al. (2019) observed a significant reduction in the rates of spontaneous preterm births in pregnant women that received a cervical pessary, they did not observe a significant reduction in composite neonatal mortality..[1] However, they did note statistically significant reductions in neonatal birth weight less than 2500g, neonatal sepsis, and NEC in the group that received cervical pessaries.[1] Conversely, Rehal et al. (2021) noted that the administration of vaginal progesterone did have a significant reduction in the rate of neonatal mortality.[2] They also noted that this intervention had a significant reduction in neonatal respiratory distress syndrome, birth weight less than 1500g, and the need for mechanical ventilation.[2] Unlike Rehal et al. (2020) and Merced et al. (2019), Shabaan et al. (2019) observed no difference in neonatal mortality and morbidity in women that received vaginal progesterone during pregnancy and the control group receiving no additional treatment.[3]

2.7 Review of Studies to Identify Potential Risk Factors/Confounders

There are many potential confounders for preterm birth such as baseline genotypes, biochemical variables, lifestyle characteristics, socioeconomic status, education level, etc., that have not been included in many previous studies and are beyond the scope of this study. It is important to consider the potential for these variables to act as confounding factors when evaluating the outcomes of the discussed studies and when analyzing the results of the proposed study. However, there are many risk factors that should be measured and adjusted for in the proposed study.

2.7.1 History of Preterm Birth

The most important risk factor that predicts preterm birth is a prior history of preterm birth.[45] The risk increases further if there was a history of more than one preterm birth.[45] If the gestational age was smaller, it further increases the risk of preterm birth.[45] For a twin gestation among mothers who have had a preterm singleton gestation, the chance of recurrence is 57% (95% CI: 51.9-61.9).[46] As twin pregnancy itself is a risk factor for preterm birth, when compounded with a prior history of preterm birth, it has a significant impact on the rate and severity of preterm birth.[45,46]

2.7.2 Maternal Risk Factors

There are notable ethnic disparities to consider with preterm birth. A meta-analysis conducted by Schaaf et al. (2012) highlighted the risk of preterm birth as higher among non-white women (odds ratio=2.0, CI:1.8-2.2) when compared to Caucasian mothers.[28] However, findings demonstrated that while the rates of preterm birth were higher among non-white women, the neonatal outcomes were better among African American mothers.[28] Maternal body mass index (BMI) is also another

important risk factor for preterm birth. Women who are extremely underweight with a BMI of < 17 kg/m^2 have a higher risk of preterm birth (odds ratio=2.4, CI: 1.4-4.2).[29] Potential reasons include macro-and micronutrient deficiencies that impair detail growth. Also, women who are obese with a BMI >34.9 kg/m^2 are also at increased risk of developing premature rupture of membranes (odds ratio=1.6, CI: 1.1-2.3).[29] In these women, the circulating inflammatory agents are suspected to increase the rate of preterm birth.[29]

Mothers who are actively smoking are also known to contribute to an increased risk of preterm births.[30] There have been many studies that have reported the risk of smoking during pregnancy with very high rates of preterm births among women. A meta-analysis demonstrated that the rate of preterm births significantly reduced following a reduction in mothers who were smoking.[31,32]

2.7.3 Excisional Cervical Procedures

Preterm birth is frequently observed among women with a short cervix.[33] The use of cervical cerclage has been present since the 1950s for women with cervical insufficiency.[34] Overall, the outcomes of prophylactic cerclage have remained unclear.[34] A study reviewed the outcomes of cerclage among women who had undergone conization.[35] While there was no significant contribution relating to age or years from conization, conization followed by cervical cerclage increased the risk of preterm birth.[35] Potential contributors include inflammation following cervical cerclage and ensuing intra-amniotic inflammation resulting in elevated preterm birth rates.[35] The risk of developing spontaneous preterm birth < 35 weeks among women who have had a history of the excisional procedure is 13%.[35] A meta-analysis conducted by Conner et al. (2014) identified the association between loop

electrosurgical excision and preterm birth.[36] Overall, there was a higher risk of presenting with preterm birth < 37 weeks following loop electrosurgical excision procedure (LEEP) (pooled relative risk= 1.60, 95% CI: 0.99-2.55), but these findings were not statistically significant.[36] Another meta-analysis conducted by Danhof et al. (2015) compared the risk of preterm birth among women who had an excisional procedure for cervical intraepithelial neoplasia prior to and while pregnant, to women with cervical intraepithelial neoplasia that remained untreated.[37] The outcomes suggested that women who were treated during pregnancy had the highest risk of preterm birth (OR=6.5, 95% CI: 1.1-3.7).[37]

A study conducted by Goldenberg et al. identified that a cervical length < 25 mm at 24 weeks was a strong predictor of preterm birth in twin pregnancies (odds ratio= 6.9, 95% CI: 2.0-24.2).[38] A meta-analysis presented interesting findings concerning cervical length and early preterm birth for twin pregnancies. A cervical length < 26 mm was shown to have a positive likelihood ratio of 9.6 (95% CI: 5.8-14.8) when screened at 20 weeks.[39] While there is are no guidelines for the benefits of screening for cervical length or cervical insufficiency, many preventive strategies are being considered for twin pregnancies.[39]

2.7.4 Uterine Anomalies

Various uterine abnormalities such as the uterine didelphys, unicornuate uterus uterine septum, and bicornate uterus contribute to preterm birth.[40] A retrospective study identified 203 women with singleton pregnancies who had a uterine anomaly. The odds of having a preterm birth increased by 5.9 (95% CI: 4.3-8.1).[40] Another systematic review identified that the rate of preterm births increased among women

with septate uteri (risk ratio=2.14, 95% CI: 1.48-3.11) and unification defects (risk ratio=2.97, 95% CI: 2.08-4.23).[41]

2.7.5 Curettage History

Women who have had a history of pregnancy loss either due to termination (abortion) or miscarriage and have been managed with cervical dilation and curettage are known to be at higher risk of developing spontaneous preterm birth (odds ratio=1.66, 95% CI: 1.14-2.42).[42] The risk rises with a higher number of curettage procedures.[43] A meta-analysis conducted by Saccone et al. (2016) identified that a spontaneous abortion (odds ratio=1.19, 95% CI: 1.03-1.37) and termination of pregnancy (odds ratio=1.52, 95% CI: 1.08-2.16) were both independent predictors of spontaneous preterm birth.[44]

2.7.6 Bacterial Vaginosis

Bacterial vaginosis is due to an overgrowth of certain bacteria in place of normal vaginal lactobacilli. A meta-analysis conducted by Leitich et al. among 30,518 indicated that bacterial vaginosis doubled the risk for preterm birth (odds ratio=2.16, 95% CI: 1.56-3.00).[47] However, the impact of bacterial vaginosis on twin pregnancy remains unclear. Another meta-analysis demonstrated a weak association of bacterial vaginosis in twin pregnancies.[38] A Cochrane review identified no impact on preterm birth rates among singleton pregnancies, high- and low-risk.[48] However, another Cochrane review concluded that treatment of bacterial vaginosis reduced the preterm birth rate among high-risk singleton pregnancies (relative risk= 0.64, 95% CI: 0.47-0.88).[49] The treatment with clindamycin before 22 weeks of gestation to mothers with

bacterial vaginosis have shown efficacy for preterm birth < 37 weeks (relative risk= 0.60, 95% CI: 0.42-0.86).[49]

2.7.7 Group B Streptococcus Maternal Colonization

A meta-analysis conducted by Bianchi-Jassir et al. (2017) estimated the risk ratio for preterm birth associated with maternal Group B streptococcus (GBS) colonization to be significant.[50] GBS colonization in mothers has been shown to increase the risk of preterm birth (odds ratio=1.98, p<0.001, 95% CI: 1.45-2.69).[50] Bacteriuria as an ascending infection is known to increase the risk of preterm birth associated with maternal GBS.[50] A retrospective cohort identified the burden of GBS at 14 weeks of gestation in singleton pregnancies. The incidence of preterm birth was significantly higher among the GBS-positive mothers (p=0.001).[51]

2.7.8 Fetal Anomalies

The risk of preterm birth for pregnancies associated with congenital fetal anomalies varies upon the type of anomaly.[52] Overall, the gastrointestinal anomaly was associated with a 2.62-fold increase in the odds of developing a spontaneous preterm birth (95% CI: 1.52-4.53).[52] After adjusting for maternal age, ethnicity, tobacco and substance use, and BMI, the risk of preterm birth increases by 4.81 fold (95% CI: 2.86-8.09) with gastrointestinal anomaly and 3.66 fold (95% CI: 1.06-12.64) for neck mass anomaly.[52]

2.7.9 Compliance with Vaginal Progesterone

Compliance is assessed by the patients returning medication packs, diaries, and self-reports. It is calculated as the total number of doses taken divided by the

expected doses. A study observed adequate compliance to be 80% or more among women taking vaginal progesterone.[53] Overall, over 82% (n=1011) of the women had a compliance of over 80% in the trial.[50] Two other studies reported a high compliance rate of 93-96% among women for gel forms of vaginal progesterone.[33,54] Another form of vaginal progesterone including oil-in-capsule was less easy to use and associated with higher vaginal discharge.[55] However, the overall satisfaction of vaginal progesterone is higher than that of intramuscular progesterone.[56]

2.7.10 Gestational Diabetes

For twin pregnancies, the risk of adverse outcomes with gestational diabetes mellitus (GDM) is known to increase.[57-59] A retrospective cohort study identified the outcomes of twin pregnancies among mothers with GDM. There was a higher risk of poor perinatal outcomes except for macrosomia.[57] The risk of preterm birth was increased by an OR of 58.82 (95% CI: 31.25-135, $p<0.0001$) among GDM mothers expecting twins.[57] Another study reported a lack of clear guidelines to manage patients with twin GDM pregnancies.[58] Overall, twin GDM pregnancies had a higher risk of prematurity and perinatal mortality.[58] There was also a higher rate of perinatal mortality among twin GDM pregnancies compared to singleton pregnancies ($p=0.001$).[58] There are poorer outcomes of GDM with twins vs. singleton GDM pregnancies.[59] These women are known to be in a high-risk group with a lack of clarity on dietary requirements, the timing of delivery, and glucose targets.[59]

2.8 Review of Empirical Literature on the Relevant Methodology of Studies

Merced et al. (2019) conducted an open, randomized, controlled trial in 132 pregnant women that had a short cervix (< 20 mm between 24 and 29 weeks of

gestation and < 10 mm between 30 and 33 weeks of gestation) and did not deliver 48 hours after an episode of threatened preterm labor. This trial was conducted between December 2010 and December 2014 in Barcelona, Spain, with ethics approval from the hospital ethics committee. Inclusion criteria for the study included a minimum maternal age of 18 years, twin pregnancies, and gestational age between 24 and 33 weeks with a short cervical length and arrested preterm labor. Exclusion criteria included women with a cervical cerclage in situ, history of a cone biopsy, active vaginal bleeding or ruptured membranes, and women with regular uterine contractions despite tocolytic agent administration. The participants (n=132) were then randomized into a pessary group (n=67) and control group (n=65). The pessary group had the insertion of a cervical pessary, while the control group received routine management. The participants were followed up on a monthly basis, with no women lost to follow up. The methodology utilized by Merced et al. (2019) had a clear aim with an appropriate and justified approach. The methodology was presented with a high quality of detail to ensure that replication was possible. It was noted that Merced et al. (2019) placed the cervical pessary in participants after 24 weeks of gestation. Hence, it is not known whether earlier placement of the cervical pessary would have resulted in a greater effect on the reduction in the rate of preterm deliveries.

The methodology employed by Rehal et al. (2021) was similar to that of Merced et al. (2019) but on a larger scale. They recruited pregnant women with twin gestations from 22 hospitals across Europe (n=1194). Inclusion criteria for recruitment included pregnant women that were 18 years or older, 2 lives fetuses at 11 to 13 weeks' scan, monochorionic diamniotic twin pregnancy, and fluency in the local language. The eligible participants were assigned 1:1 to an intervention group and a control group with a simple computer-generated block. The intervention group

received a progesterone capsule to be vaginally administered while the control group received a placebo capsule to be vaginally administered that was the same size and shape as the progesterone pill. The intervention was commenced between 11 to 13 weeks of gestation. The method differed from Merced et al. (2019) at this point, as the inclusion of a placebo allowed for double-blinding in the study. All the participants, investigators, pharmacists, etc. were blinded to the allocation of the participants until the end of the study. All participants were asked to administer one vaginal capsule 300mg twice daily until 34 weeks' gestation or in the event of an unexpected early delivery. All participants were followed up once every two weeks. The methodology outlined by Rehal et al. (2021) was very detailed to allow for future reproduction. No concerns were noted on the analysis of the method outlined by Rehal et al. (2021).

Shabaan et al. (2018) conducted an open-label, randomized, controlled trial across three tertiary care centers in Egypt between 2015 and 2017. The inclusion criteria included pregnant women at 28 weeks of gestation that were naturally conceived, no known major fetal abnormalities, dichorionic diamniotic twins, and uncomplicated pregnancy. In total, 158 women were recruited to participate in the study, of which 18 were excluded due to not meeting the eligibility criteria. The participants (n=140) were randomly assigned 1:1 into two groups, an intervention group, and a control group. They determined that both groups had comparable baseline characteristics. The intervention group received 400mg once daily at bedtime from 28 weeks' gestation. The control group received standard care. The pregnant women were followed up once every two weeks until delivery. The primary outcome of the study was the rate of premature birth prior to 37 weeks' gestation. Rehal et al. (2021) had conducted a study to observe the effects of vaginal progesterone on reducing preterm rates in twin pregnancies with administration from 13 weeks'

gestation to 34 weeks' gestation. They had determined that progesterone administration had resulted in a reduction in preterm rates before 24 weeks' gestation, but did not have a statistically significant effect after 24 weeks. Thus, the lack of effect that was noted by Shabaan et al. (2018) may be due to the late administration of progesterone, as their methods commenced the trial from 28 weeks onwards. An earlier administration of progesterone, as was conducted in the methods by Rehal et al. (2021), may have resulted in more significant results. Additionally, the sample size in the study conducted by Rehal et al. (2021) was much larger than that of Shabaan et al. (2018) (n=1194 vs. n=140). This larger sample size would have allowed for statistically significant results to be discovered, whereas associations may have been missed in the analysis conducted by Shabaan et al. (2018). Furthermore, a larger progesterone dose was used in the methods employed by Rehal et al. (2020) compared to Shabaan et al. (2018) (300 mg twice daily compared to 400 mg once daily). This larger dose of progesterone may have had a greater therapeutic effect and thus had a greater effect on preterm labor rates and postnatal outcomes. This would explain the positive effect that Rehal et al. (2021) observed in neonatal mortality and morbidity rates in women that received progesterone, compared to no effect in the study conducted by Shabaan et al. (2018). Rehal et al. (2021) also noted that progesterone administration reduced preterm birth rates in women before 24 weeks' gestation compared to no effect in the study conducted by Shabaan et al. (2018). A strength of the study conducted by Shabaan et al. (2018) was their analysis of the characteristics of each group to ensure that bias due to demographical differences was not missed.

2.9 Review of Empirical Literature to Identify Limitations and Possible Risk of Bias

The study conducted by Merced et al. (2019) contained a sample size of 132 participants which is small and could raise questions about the validity of the results. Also, the sample size allowed for a power of 80% which is on the lower end of the acceptable spectrum and gives rise to the potential for a type II error. Hence, there is a possibility that the study conducted by Merced et al. (2019) may have had false clinical implications. Furthermore, Merced et al. (2019) did not calculate a p-value for their results to demonstrate the statistical significance of their data. As opposed to Merced et al. (2019), Rehal et al. (2021) included a placebo in their control group that allowed for double-blinding in their study. Thus, they had a reduction in their level of potential bias compared to Merced et al. (2019). Rehal et al. (2021) did calculate a p-value for their results, but was not statistically significant for their primary outcome ($p=0.17$). Furthermore, the large sample size utilized by Rehal et al. (2021) further ensured that the study had a low risk of bias and was generalizable to the population. Another limitation of Rehal et al. (2021) was that unlike Merced et al. (2019), they did not consider confounding factors such as cervical length in their analysis of their data. This may negatively influence the external validity of their results.

Unlike Merced et al. (2019) and Rehal et al. (2020), Shabaan et al. (2018) included only women that were naturally conceived as part of their inclusion criteria. Women that undergo artificial reproduction are at an increased risk of miscarriage and preterm birth. Thus, there is a potential for bias in the studies conducted by Merced et al. (2019) and Rehal et al. (2020) if there was a greater number of women that conceived through artificial reproduction in one group compared to the other. Although randomization is thought to diminish this risk, there is still a chance that

uneven allocations can occur that may skew results. Thus, it is essential to conduct an analysis of the characteristics of each group to ensure that demographical factors do not have an impact on the validity of the results. This analysis was not conducted by Merced et al. (2019) and Rehal et al. (2020).

2.10 Conclusion

Thus, previous studies have investigated the effects of monotherapy with cervical pessaries and vaginal progesterone administration on the effects of preterm birth rates and neonatal mortality and morbidity in twin gestations. The three studies chose to be reviewed in depth have demonstrated that the administration of cervical pessary resulted in a reduction in preterm birth rates. However, there was a mixed result noted in investigations on the effect of using vaginal progesterone on the rates of preterm births. Rehal et al. (2020) noted that the use of vaginal progesterone reduced the rates of preterm birth prior to 24 weeks' gestation, but did not influence preterm birth rates between 24 to 38 weeks' gestation in twin gestation. Conversely, Shabaan et al. (2018) found no association between the use of vaginal progesterone and preterm birth rates in twin gestation. The difference in these results may be due to disparities between the methodology employed by these two studies. It was further noted that the use of cervical pessaries did not have an effect on neonatal mortality but did reduce the incidence of morbidity. Rehal et al. (2020) observed a reduction in both neonatal mortality and morbidity with the use of vaginal progesterone during pregnancy. In contrast, this reduction was not observed by Shabaan et al. (2018) who found no association between neonatal mortality and morbidity with the use of vaginal progesterone. Again, this difference may be related to the differing methodology employed by these two studies.

To date, there has been no study that has investigated the effect of combined cervical pessaries and vaginal progesterone administration on preterm delivery rates in twin gestation. Although cervical pessaries have been found to reduce preterm delivery rates in this demographic and can be used as a potential prophylactic intervention, their effect may be amplified with the use of vaginal progesterone. Furthermore, Merced et al. (2019) investigated the effect of cervical pessary insertion on preterm rates in twin pregnancies, but had this device inserted after 24 weeks of gestation. Thus, it is unknown what effect an earlier administration of this device would have on preterm delivery rates. Hence, this proposed study aims to fill this gap in the available literature by determining the effects of the early insertion of combined cervical pessary and vaginal progesterone on preterm delivery rates and neonatal mortality and morbidity in twin gestation. The insertion of combined progesterone and cervical pessary has been found to reduce the rates of preterm pregnancies in singleton gestations by 10 to 20%. It is estimated that the use of these interventions in twin gestations will have a similar rate of effect.

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CHAPTER 3: STUDY METHODS

3.1 Study Design

This is a prospective multicenter, open-label randomized control clinical trial to compare combined vaginal progesterone and cervical pessary placement in the late first trimester (11 to 14 weeks' gestation) in DCDA twin gestations until 37 weeks and 6 days' gestation or birth vs. standard care on the rate of preterm births. The pregnant participants will be randomly assigned (1:1) to a **Control Group** (no intervention, standard management) versus a **Treatment Group** (vaginal progesterone and the Arabin cervical pessary placed in late first trimester in twin gestations). The randomization will be done using a computer-generated system with balanced blocks consisting of 20 patients in each block. We will also administer a questionnaire and do an analysis of the participants' electronic medical records (EMR) to track their medical histories.

3.2 Study Setting and Population

Patients will be recruited from the twelve sites of Maternal-Fetal Medicine Unit Centers and sub-sites within the Maternal Fetal Medicine Units (MFMU) Network. The primary research will be performed at the Yale Maternal Fetal Medicine Center at the Yale New Haven Hospital (YNHH). It is imperative to have a large study consortium to ensure an adequate sample size for statistically significant results. It is estimated that approximately 160,000 deliveries are completed annually within the MFNU Network.[1] Thus, MFMU clinical centers will provide a participant pool to conduct a large clinical trial that focuses on preterm birth in multiple gestations with an emphasis on establishing potential interventions.[1] Furthermore, it is expected that the results obtained from participants recruited from the MFMU Network will be

generalizable given that these patients are not unique in their demographic characteristics.[1]

The participant population in this study is pregnant individuals with DCDA twin gestations that are being treated by the obstetric team in the MFMU Network Centers and sub-sites. An invitation will be sent to the MFMU centers prior to the commencement of the study.

Inclusion criteria will include DCDA twin gestation; gestational age between 11 to 14 weeks of gestation at the time of enrolment; participant age 18 years or older; no know chromosomal abnormality; normal cell-free fetal DNA testing; no known or suspected fetal structural abnormalities; uncomplicated pregnancy.

Exclusion criteria will include monochorionic diamniotic twin gestation; triplets or higher-order pregnancies; history of prior preterm delivery; smoking ≥ 10 cigarettes per day; alcohol or illicit drug consumption; singleton gestation; cervical insufficiency; known uterine malformations; history of hepatic dysfunction or gestational cholestasis; history of thromboembolic disease; recent (within one year) or current malignancy; vaginal bleeding; difficulty for follow-up; cervical cerclage inserted prior to the study; contraindication to treatment with progesterone; women with progesterone and peanut allergies (given that progesterone supplements may contain traces of peanut)

3.3 Subject Protection and Confidentiality

To ensure the safety of participants and compliance with ethical requirements, ethics approval will be obtained from the Intuitional Review Board prior to the commencement of the study. Given that the study involves vulnerable populations (pregnant individuals and unborn fetuses), it is essential that ethics approval is cleared

by the Institutional Review Board. To ensure that all necessary steps are completed prior to the submission for ethical clearance, the online Research Ethics and Safety Checklist for Undergraduate Experiments (RESCUE) form will be completed. It was determined that the CITI Human Subjects Research training module, Clearance for Human Subjects Ethical Research (CHSER), Participant Information Sheet (PIS), Informed Consent Form (ICF), and Fieldwork Safety Plan (FSP) must be completed. Following this, an ethics approval document and risk assessment form should be conducted. All forms will be sent to the research supervisor for review and clearance prior to sending to the College Ethics Review Committee (CERC) at Yale. After any necessary changes to the documents as per the CERC are completed, the documents can then be submitted to the Institutional Review Board for a final ethics clearance.

To ensure the confidentiality of all participants, the HIPAA guidelines will be strictly adhered to by all researchers involved in the study. The patient health information will be limited only to staff that has a direct need to access the information as part of their role in the study. Furthermore, all staff involved in the study will undergo proper medical ethics and HIPAA training. Jotforms will be used as an online HIPAA-compliant tool to allow patients to input their health information through a secure online form. The electronic transfer of patient information will be avoided unless deemed necessary. If electronic transfer is required, then patient information will undergo end-to-end encryption prior to transfer to ensure it remains secure. After the collection of all data, patient information will be de-identified prior to analysis to ensure that information remains confidential. Furthermore, trial coordinators will ensure quality control screening, verification of protocol adherence, and overseeing data handling.

3.4 Recruitment

We will use a consecutive sampling method for the recruitment process over a 6-month period. All individuals with DCDA twin pregnancies attending the MFMU Centers before 14 weeks' gestation will be considered for inclusion in this trial, provided that they do not meet any of the aforementioned exclusion criteria. The site coordinators at the MFMU locations will be asked to provide potential participants with written information about the study and the trial. Those who agree to participate will provide the recruitment team with the authority to release their contact information to the research team. Prior to inclusion in the study, eligible participants will be screened by midwives or gynecologists to ensure they are healthy and able to participate in the study. All pregnancies will be dated by crown-rump length during the first trimester, and chorionicity will be determined. Eligible participants who agree to participate in the study will then be provided with a fully detailed participant information sheet and with the investigators contact information for any queries or questions before agreeing to participation. They will then be provided with a written informed consent form to sign. An incentive of a $100 gift card will be given to each participant that completes the trial. After signing the informed consent, participants will have the option to drop out of the study for any reason and at any time. Furthermore, providers as part of the investigative team will have the option to withdraw the participants from the study if urgent medical reasons arise. Based on the intention to treat principle, all subjects will be included in the analysis.

3.5 Key Study Variables, Measures, and Operationalization

3.5.1 Study Intervention

At the time of recruitment, we will obtain a detailed medical history for all participants, including their medical, personal, obstetric, and menstrual history through both EMR and questionnaires. The gestational age will be estimated if there is a reliable date available. If this is not available, then the providers will obtain the gestational age using a first-trimester ultrasound crown-rump length. All participants will receive their usual antenatal care for multiple gestations, including iron and calcium supplements. The eligible participants (pregnant individuals) will be randomly allocated in a 1:1 to two groups: Group 1 (Cervical Pessary and Progesterone Group) will have the insertion of an Arabin cervical pessary between 11- and 14-weeks' gestation, followed by administration of 400 mg of vaginal natural progesterone once daily. These interventions will continue until 37 weeks' gestation. Group II (control group) will receive no additional treatment beyond standard care. The independent variable in this study will be the combination therapy of the cervical pessary and vaginal progesterone. The dependent variable will be the mean gestational age at delivery and neonatal morbidity and mortality. Control variables include standard antenatal care for multiple gestations. The primary outcome of interest is the difference in the rate of preterm birth between the intervention group and the control group. The secondary outcomes include overall gestational age at delivery; preterm birth before 35, 34, 32 and 28 weeks of gestation; cervical length changes throughout pregnancy (measured in millimeters via transvaginal ultrasound); birth weight; APGAR score at birth and five minutes after; admission to the NICU and the time spent there if needed (of one or both of the twins); and composite neonatal outcomes

of morbidity and mortality (the occurrence of any of the following events: RDS, IVH, sepsis, NEC and death before hospital discharge).

3.5.2 Follow-up

Participants in both the treatment and control groups will be followed up during their antenatal visits at the multicenters every week until they deliver. During each visit, they will be asked to report symptoms of preterm labor such as abdominal colic, heaviness, cramps, and sudden expulsion of a gush of fluid. They will also be asked about compliance and any notable side effects from use of the daily vaginal progesterone. During these weekly visits, the participants will also undergo an ultrasonography scan to evaluate the wellbeing of the fetuses. Any complications, such as intrauterine growth restriction, placental abruption, preeclampsia, etc. will be noted during these visits. At the time of delivery, neonatal birth weight, delivery data, referral to neonatal care unit, and any intrapartum or postpartum events will be recorded. The women and neonates will continue to be followed up on a monthly basis for 12 months after delivery to monitor for neonatal morbidity and mortality rates.

3.6 Methodology Considerations

There are a number of confounding variables in this study, such as cervix shape; prior cervical operations, such as cone biopsy, LEEP, etc.; compliance to vaginal progesterone; fetal abnormalities; miscarriage; bacterial vaginosis during pregnancy; GBS carrier mother; premature rupture of amniotic membranes; GDM; preeclampsia; and thromboembolic event. Certain characteristics pertaining to maternal factors such as age, ethnicity/race, educational level, socioeconomic status, BMI, or mental health status may also have confounding effects. These confounding

variables will need to be taken into consideration when completing the data analysis. Furthermore, the participants' mental health status may predispose her to cervical disease that will be accounted for when analyzing the data. For this reason, multiple logistic regression analyses will be conducted in the data analyses period to control for any or all the listed confounding variables.

3.7 Blinding of Interventions/Outcomes

Due to the nature of this study, the participants and the medical providers inserting the cervical pessary will not be able to be blinded to their group allocations. Given that this an open-label clinical trial YNHH Investigational Pharmacy will be informed to distribute regular packets of progesterone to the intervention and standard of care group as applicable. All participants, pharmacists, investigators, and other research personnel will be informed of the nature of the study design, and they will not be blinded to the intervention during treatment, data collection, and data analysis. The data analysis will be performed once the data has been charted, cleaned and readied.

3.8 Assignment of Intervention

The randomization will be performed through a computer-generated random assignment system with balanced blocks to ensure equal allocation to each group. After the allocation to groups, the control group will be asked to attend weekly follow-up appointments at the Yale Maternal Fetal Medical Center to receive their standard care and monitoring of maternal and fetal health. The intervention group will be booked into an urgent appointment to have the insertion of a cervical pessary prior to 14 weeks of gestation. At this appointment, they will receive 7 doses of vaginal

progesterone capsules to administer every night for the next week until their next follow-up. The subjects will receive instructions and education on how to self-administer the vaginal capsule. The subjects will receive a week supply of daily vaginal progesterone capsules at each weekly follow-up appointment.

3.9 Adherence

Because one intervention modality (i.e., progesterone) is self-administered based on the subject's intake location, the adherence protocol will be assessed by having the participant complete a log of medication taken and any missed doses and to return the empty packet of the previous supply. We will consider the compliance to be adequate for inclusion in the data analysis if the participant completed 80% or more of the prescribed doses, which is in line with the standard cut-off for compliance in any clinical trials. Furthermore, the cervical pessary will be monitored by the primary clinician to note any deviations subjective to the patient. We will provide appointments at various time to fit the participants' schedule and transportation for those who need it. If a participant is unable to make an appointment, their next week's vaginal progesterone packet can be ordered to their local pharmacy to pick up.

3.10 Monitoring for Adverse Events

While adverse events may occur in this clinical study, they are expected to be extremely rare. The primary investigator of the study will be obliged to notify the subjects and the medical research ethics committee if any unforeseen negative outcomes, adverse effects, or disadvantages secondary to participation in the study are observed that are greater than initially noted in the research proposal. In this case, the study would be suspended until it has been reviewed by the medical research ethics

committee. An adverse event is defined as an undesirable experience that occurred to a participant in the study, regardless of whether this is related to the interventions. Adverse events that may occur due to progesterone administration include fluid retention/bloating, breast pain, drowsiness, depression, hot flushes, dizziness, vaginal discharge, abdominal pain or cramping, and more.[2] Due to the vaginal administration method of progesterone, there are limited levels of progesterone that reach systemic circulation in this study, compared to other routes of administration. The main adverse event associated with cervical pessaries is an increase in vaginal discharge.[3] All participants will be informed of the potential adverse effects prior to acquiring written consent. Furthermore, all participants will be asked to report experience any adverse events at each weekly follow-up visit. These adverse events will be monitored until the situation becomes stable or the adverse event has abated. Additional tests, medical procedures, or referrals to medical specialists/general physicians will be performed as indicated.

A serious adverse event is defined as a medical occurrence or event that results in a life-threatening condition, death, permanent or significant disability, hospitalization, a birth defect or congenital abnormality, or is a new event in the trial that is likely to affect the safety of participants. In the case of a serious adverse event, the study will be suspended until a further review is conducted and the ethics committee that granted approval will be informed.

3.11 Data Collection

Data will be collected at randomization and at each weekly follow-up appointment at the Yale Maternal Fetal Medicine Center and other MFMU Network centers using a standard questionnaire that will be provided to each medical provider

involved. All centers will list basic demographic and clinical characteristics data obtained from the EMR and questionnaires. This provide information on the maternal racial group/ethnicity, age, educational level, socioeconomic status, mental health, BMI, or any substance use history. These questionnaires are expected to limit potential confounders, and the research team will have a better estimate in the management of confounders as the study progresses. This will be recorded using the Epi Info software (Centers for Disease Control and Prevention) and documented in the subject's study profile. The questionnaire will include questions about any adverse events experienced by the participant, results of ultrasonography, any symptoms of preterm labor, etc. If the participant is unable to attend the clinic for any follow-up appointment, data will be collected through a telephone interview where they will be asked to answer the questionnaire. We will also document cervical length changes throughout pregnancy (measured in millimeters via transvaginal ultrasound) and determine if the mother has cervical insufficiency.

After the participant arrives for delivery we will review their medical record to acquire data on the labor, delivery, and any complications that may have arisen for the participant or neonates. The gestational age at delivery and whether or not it is preterm (< 37 weeks) will be recorded (primary outcome data), with the division of groups in preterm birth before 35, 34, 32, and 28 weeks of gestation (secondary outcome data). We will assess the birth weight of the baby as part of the standard operating protocols, hence no, hence no additional personnel will be required. The nursing team will ensure that the APGAR score at birth and five minutes after is charted correctly. Admission to the NICU and the time spent there if needed (of one or both twins) will be noted with yes and no options (dichotomous data). A set of lists for composite neonatal outcomes of morbidity and mortality including 1. RDS, 2. IVH, 3. Sepsis, 4. NEC and

5. Death before hospital discharge will all be enlisted as dichotomous variables by the research assistants which will be regularly cross-checked and finally verified by the research heads at the different centers

The participants will then attend a monthly follow-up appointment for 12 months after delivery. At the first follow-up appointment after delivery, they will be asked about their delivery and labor details and whether any complications were experienced to ensure that information is not missed. Additionally, they will be asked screening questions for the neonates' health status, including regular development questions, any symptoms of infections that may have been noted, and any concerns the mother has. The neonates' weight, length, and head circumference will also be measured at each follow-up to monitor growth.

3.12 Sample Size

Sample size will be calculated based on a 20% reduction of the reported 60% preterm birth rate in twin pregnancies before 37 weeks' gestation.[4] The calculation was done based on Cox Proportional Hazard Regression with an α error of 0.05 and a β error of 20% (i.e. with a power of 80%). Accordingly, the sample size was calculated to be 97 participants in each arm, but when accounting for a maximum loss to follow-up of 25% that equates to 122 participants in each arm. The effect size chosen was 0.1 in order to get a larger sample size because the relationship between the primary outcome of gestational age and the independent variable of cervical pessary and progesterone is more likely to be detected with a larger sample size. Sample size calculation was done using G*Power, version 3.1.9.6 for macOS.[5]

3.13 Analysis

Statistical analysis will be carried out on an intention-to-treat basis, thus all participant will be included in the results. No interim analyses will be performed. In cases of a strong positive effect of the combination intervention, the trial will proceed. Negative effects will be detected by a data safety monitoring committee. Outcomes will be evaluated using Cox Proportional Hazard Regression model, as well as with secondary outcomes looking at gestational age at birth < 35, < 34, < 32, and < 28 weeks' gestation. Student t-test will be used for continuous outcomes of secondary variables (birthweight, cervical length changes, etc.), and Chi-Square analysis will be used for categorical data of secondary outcomes.

3.14 Timeline and Resources

The study timeline includes 6 months for recruitment and baseline data collection, 12 months to assess outcomes, and 6 months for data analysis. It is estimated that the research team at the Yale Maternal Fetal Medicine Center at YNHH will include one principle investigator, one project coordinator, one project manager, medical providers as needed that will provide antenatal care to the participants, administer interventions, and collect and upload weekly follow-up data, one data analyst, 3-4 research assistants, five data compilers, and five data analysts. Medical offices as needed per site and one procedure room will be required at the Yale Maternal Fetal Medicine Center to conduct follow-up appointments and insert cervical pessaries. Cervical pessaries and vaginal progesterone capsules will need to be ordered. Given that all 12 MFMU Networks sites may partake in the study, there will be up to an additional 11 site coordinators, and medical providers as needed per site.

The intake surveys coordinators, and data collection and entering will be led by the resident physicians or medical fellows. Providers will be compensated for their time.

Additionally, equipment for the insertion of cervical pessaries, such as speculum, will be required. Subscriptions for the online data tools that will be used in this investigation will need to be organized. This includes Jotforms and the SPSS software package. Overall, it is expected that this study will span across a two-year period. It is expected to start in January 2022 and be completed by January 2024.

3.15 Accounting for Loss to Follow-Up

The primary strategy that will be utilized to avoid bias due to loss to follow-up and consequent missing data will be initial prevention. This will be achieved by careful design of the study, high level of training of staff, developing mechanisms to ensure that participants can be easily contacted, and implementing data quality procedures. Each arm of the study will include 122 participants, rather than the calculated 97 participants, to account for a maximum loss to follow-up of 25%. The secondary strategy to avoid loss to follow-up bias is to use an intention-to-treat methodology in analysis. Thus, even if participants drop out or are lost to follow-up, they will be included in the analysis of the data. Additionally, the rate of loss to follow-up and level of missing data will be reported in the final paper to ensure transparency.

3.16 References

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CHAPTER 4: CONCLUSION

4.1 Advantages and Disadvantages

There are several advantages and disadvantages of the proposed study and its methodology that may impact the reliability of the data to be obtained. The most central strength of this study is the notion that we will contribute to existing literature since no study has been done that looks at early use of vaginal progesterone, in combination with cervical pessary. Other strengths of the study include the randomized, controlled design, which is expected to minimize bias, including bias from confounding factors. Furthermore, the randomized nature of the study is expected to enhance the generalizability of the study and combat any confounding variables. Additionally, there will only be a limited number of staff that will be involved in the insertion of the cervical pessary, which should have a positive impact on the validity of the study.

The large sample size is expected to have a positive impact on the power and statistical significance of the results. However, this is also seen as a limitation since it is unknown whether obtaining such a large number of participants in a 6-month time period given the narrow inclusion criteria is feasible. The ultimate sample size may be significantly smaller than desired if a substantial number of viable participants are not found. Thus, the study may have to span for a longer period of time than intended to ensure adequate power and statistical significance. This will be addressed by informing the participant that they will receive optimal care and surveillance throughout their pregnancy, which will benefit themselves and the twins. We will also incentivize the participants with the $100 gift card upon completing the trial.

A limitation of the study is that the open nature of the study with no blinding or masking may result in the introduction of bias. This unfortunately cannot be

avoided due to the nature of the interventions. However, given that the primary endpoint of the study is an objective variable (gestational age at delivery), it is believed that the non-masked nature of the trial should not result in a significant level of bias. A factor that may compromise the external validity of the study is that the majority of women with twin pregnancies will have conceived from assisted reproductive technology. This consequently may have a negative impact on the generalizability of the results.

A potential limitation is the lack of previous studies that have investigated the effects of early implementation of progesterone and cervical pessaries on the rates of preterm births in multiple gestations. As a result, the data cannot be compared to empirical evidence. However, futures studies would be able to reproduce this study and see if they yield different results. Also, a percentage change reduction in preterm births that will be expected to arise from the interventions proposed in this study was not based on previous studies and findings. Similar studies that were conducted in singleton pregnancies have demonstrated an approximately 20% reduction in the rate of preterm births. Thus, it is expected that the results of this study will mimic these results and consequently lead to a 20% reduction in the rate of preterm births in twin gestations with the use of progesterone supplementation and cervical pessary insertion.

4.2 Clinical and/or Public Health Significance

The use of either vaginal progesterone or cervical pessary is known to successfully delay preterm birth in singleton pregnancies, but no studies have provided statistically significant data about an intervention that prevents or delays premature birth in twin pregnancies.[1,2] Therefore, there is no established standard of care or reliable treatment to increase the average gestational age and lower the rates of

preterm birth in twin pregnancies.[3] With the trend of increasing incidence of twin pregnancies, there are higher rates of preterm birth and higher rates of morbidity and mortality associated with preterm birth in this population compared to singleton pregnancies.[4] Premature birth is responsible for greater than 50% of all neonatal deaths for multiple gestations; an effective treatment is needed now more than ever.[5] If this intervention proves to be effective it can lead to a new standard of care and significantly decrease the morbidity and mortality that affect the majority of twin pregnancies.

Because most studies look for a 50% decrease in preterm birth between the intervention and control group, a goal of a 20% decrease will give this study a better chance at success.[6-10] The study is unique since it will include a multitude of important factors that can help determine if the intervention truly is more effective than those used in previous studies, even if the rate of preterm birth is not significantly reduced. Some of these important secondary outcomes are overall gestational age; level of preterm birth based on if the twins are < 35, < 34, < 32, and < 28 weeks' gestation at birth; birth weight; cervical length during pregnancy; APGAR score; admission to the NICU and the time spent there if needed; and composite neonatal outcomes (the occurrence of any of the following events: RDS, IVH, sepsis, NEC and death before hospital discharge).[10,11] Discovering statistically significant improvements in these outcomes could also establish this intervention as the treatment of choice for preventing morbidity and mortality in twin gestations that are premature.

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Appendix A: IRB Consent Form

Instructions and invitation to participate:
You are invited to participate in our study that aims to investigate the effect of early administration of vaginal progesterone and cervical pessaries on the rates of preterm births in twin gestations. Currently, twin gestations account for approximately 60% of all preterm births. This places these babies at a great risk of potential harm to their health. We aim to reduce this risk by devising an intervention that will reduce the rates of twin babies that are born preterm. In this study, you will be randomly allocated to a group that either receives combined vaginal progesterone and cervical pessary, or to a group that receives no additional interventions. We will then analyze your pregnancy outcomes and the outcomes of your babies and compare the rates of preterm births and the babies' health status. In order to decide whether you want to participate in this study, we will provide you with an additional form with all of the risks and benefits of participation. Additionally, we invite you to have a consultation with our primary investigating team so that you are openly able to ask any questions or raise concerns. By providing you with this detailed information, we want to make sure that you make an informed decision regarding your participation.

Economic Considerations
There will be no monetary benefits to participating in this study.
Audio/Video Recording
All consultations will be recorded to assist with analysis of our data. These will be kept confidential and will only be used by the analysts in the study. No distribution of these videos will occur and they will be destroyed on the completion of the study.

Please sign below if you are willing to have this interview recorded *(specify audio or video)*. You may still participate in this study if you are not willing to have the interview recorded.

☐ I do not want to have this interview recorded
☐ I am willing to have this interview recorded

Signed: _____

Date: _____

Disclaimer:

- *A video recording in which the your name, likeness, image, and/or voice will be included will be recorded*
- *By signing this form, you give us the right to make, use and publish Recordings in whole or in part in media forms now known (such as film, slides, and digital audio) or developed in the future. This includes the right to edit or duplicate any images/recordings*
- *There will be NO reproduction, distribution, performance, or display of images/recordings*
- *By signing this form, you relinquish the rights to inspect or approve the finished product or printed/published matter that uses the images/recordings or versions of the images/recordings;*

If you are injured by this research
In the event that any research-related activities result in an injury, treatment will be made available including first aid, emergency treatment, and follow-up care as needed. Cost for such care will be billed in the ordinary manner to you or your insurance company. No reimbursement, compensation, or free medical care is offered. If you think that you have suffered a research-related injury, contact *[PI name]* right away at *[insert phone number]*.

Privacy/Confidentiality
The information regarding your involvement with the study will be recorded in your Electronic Medical Record. From there, this information will be accessible by your providers who participate in managing your health. Your medical records will be accessible by the investigators of this study. Additionally, it is possible that the Food and Drug Administration (FDA) may need to review all records of participants in the study, however these personnel are bound by the rules of confidentiality to not disclose your name or information to others. Additionally, the Yale Human Research Protection Program Committee may access study records to conduct an internal audit. In this case, all members of the committee are required to keep all information and records confidential. All your identifiable information will remain confidential for the entire duration of the study. Furthermore, your data will be de-identified prior to analysis to ensure confidentiality. Regardless, all study personnel are legally bound by the rules of confidentiality and breaches of these rules will have severe legal repercussions.

Data Sharing
De-identified data from this study may be shared with the research community at large to advance science and health. We will remove or code any personal information that could identify you before files are shared with other researchers to ensure that, by current scientific standards and known methods, no one will be able to identify you from the information we share. Despite these measures, we cannot guarantee anonymity of your personal data.

Clinical Trial
This study is classified as a clinical trial and will be registered online at http://www.ClinicalTrials.gov. The website will not include any information that can identify you, but will include a summary of results once the research is completed. You can search this publicly-available website at any time.

Taking part is voluntary
Your involvement is voluntary, and you may refuse to participate before the study begins, discontinue at any time, or skip any questions/procedures that may make you feel uncomfortable, with no penalty. It will not harm your relationship with the investigators in this study or with your doctors/medical professionals. If you withdraw, there will be no ongoing collection of data. However, data that was previously collected may still be used in the analysis of the results to ensure that the integrity of the study is maintained.

Withdrawal by investigator, physician, or sponsor
The investigators, physicians or sponsors may stop the study or take you out of the study at any time should they judge that it is in your best interest to do so, if you

experience a study-related injury, if you need additional or different medication/treatment, or if you do not comply with the study plan. They may remove you from the study for various other administrative and medical reasons. They can do this without your consent.

If you have questions
The main researcher conducting this study is *[principal investigator's name]*, a *[professor, graduate/undergraduate student, etc.]* at Yale University. Please ask any questions you have now. If you have questions later, you may contact *[principal investigator's name]* at *[email address]* or at *[phone number]*. If you have any questions or concerns regarding your rights as a subject in this study, you may contact the Institutional Review Board (IRB) for Human Participants at 607-255-5138 or access their website at http://www.irb.cornell.edu. You may also report your concerns or complaints anonymously through Ethicspoint online at www.hotline.cornell.edu or by calling toll free at 1-866-293-3077. Ethicspoint is an independent organization that serves as a liaison between the University and the person bringing the complaint so that anonymity can be ensured.

You will be given a copy of this form to keep for your records.

Statement of Consent
I have read the above information, and have received answers to any questions I asked. I consent to take part in the study.

Your Signature_____
Date_____

Your Name (printed)_____

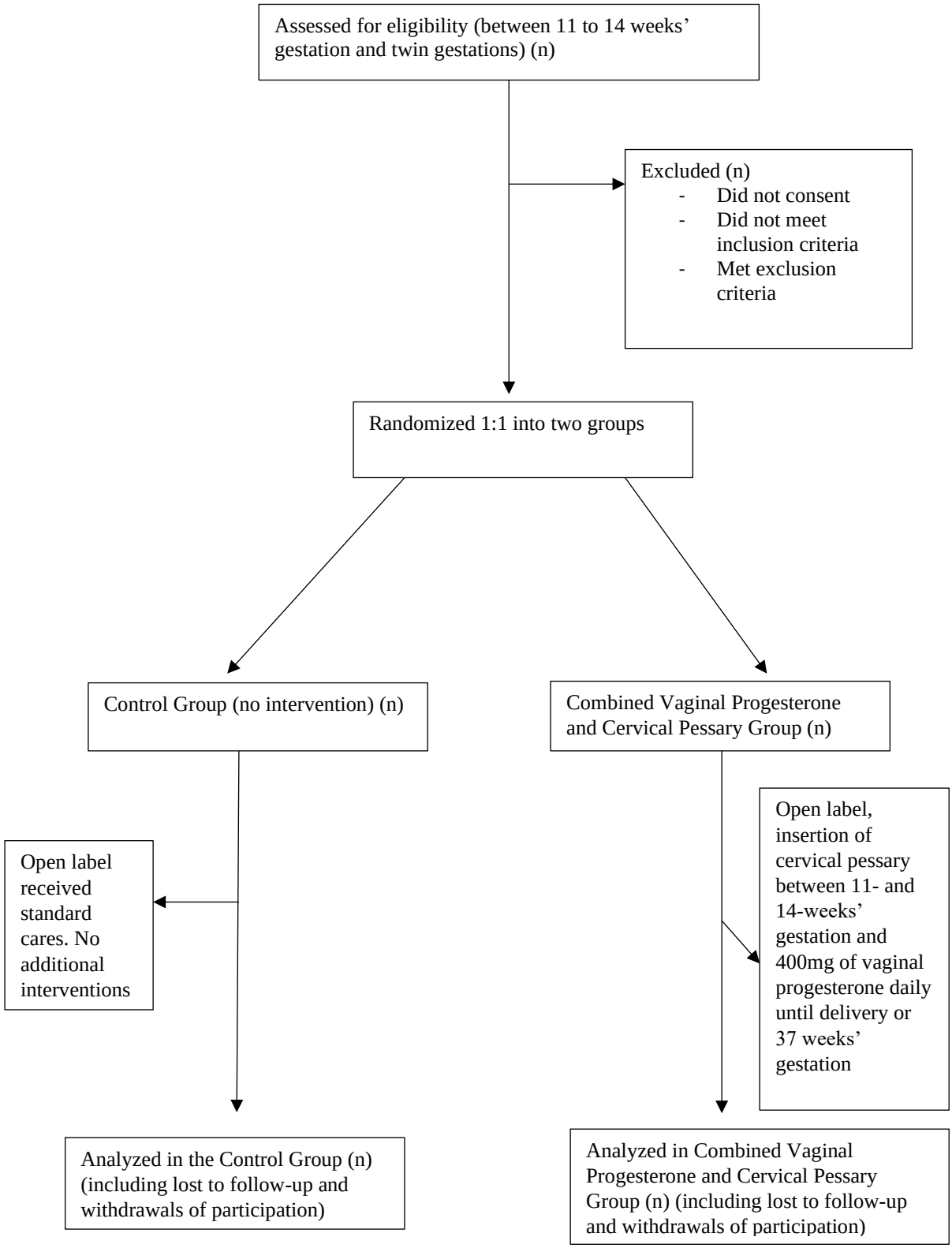
Signature of person obtaining consent_____
Date_____

Printed name of person obtaining consent_____

This consent form will be kept by the researcher for at least five years beyond the end of the study.

Reference:
1. IRB Consent Form Templates | Cornell Research Services. Researchservices.cornell.edu. https://researchservices.cornell.edu/forms/irb-consent-form-templates. Published 2021. Accessed July 2021.

Appendix B: Trial Profile



Appendix C: Maternal Demographic and Medical History Data Collection

Table 3: Maternal Demographic and Medical History Data

Maternal Physical Characteristics and Demographics	**Control Group (n=122)**	**Combined Vaginal Progesterone and Cervical Pessary Group (n=122)**
Maternal age at time of enrolment		
Maternal gestational age at time of enrolment		
Education Level		
Ethnicity		
Body-Mass Index during pregnancy		
Gravidity and Parity		
Marital Status		
Household Monthly Income		
Behaviors During Pregnancy	**Control Group (n=122)**	**Combined Vaginal Progesterone and Cervical Pessary Group (n=122)**
Alcohol Consumption		
Actively Smoking		
Passively Smoking		
Highly Active Level		
Folic Acid Supplementation		
Illicit Drug Use		
Screening		
Pregnancy Outcomes	**Control Group (n=122)**	**Combined Vaginal Progesterone and Cervical Pessary Group (n=122)**
Pregnancy-induced hypertension		
Gestational diabetes mellitus		
Anxiety		
Depression		
GBS colonization		
Eclampsia/pre-eclampsia		

Mullerian anomaly		
Bacterial vaginosis		
HELLP Syndrome		
Abortion		
Spontaneous Abortion		

Appendix D: Pregnancy Outcomes (Detailed)

Table 4: Pregnancy Outcomes for Mother and Neonates

	Control Group (n=122)	**Combined Vaginal Progesterone and Cervical Pessary Group (n=122)**	**95% Confidence Interval**	**Relative Risk**	**P-value**
Delivery Outcomes					
Total Deliveries					
<28 weeks					
28 – 30 weeks					
30 to 32 weeks					
32 to 34 weeks					
34 to 36 weeks					
>37 weeks					
Preterm < 37 weeks					
Full-term					
Spontaneous Deliveries					
<28 weeks					
28 – 30 weeks					
30 to 32 weeks					
32 to 34 weeks					
34 to 36 weeks					
>37 weeks					
Preterm < 37 weeks					
Full-term					
Induction Required					
Post-term					
PROM					
Placental abruption					
Hypertension					
Gestational Diabetes					
Oligohydramnios or IUGR					
Fetal Distress					
Delivery Mode					

Natural Vaginal Birth					
Operative Vaginal Birth					
Caesarean section prior to labor					
Caesarean section after onset of labor					
Neonatal Outcomes					
Live births					
Death prior to discharge from hospital					
Admission to NICU					
APGAR Score at 1 minute					
APGAR Score at 5 minutes					
Respiratory distress syndrome					
Intravascular hemorrhage					
Sepsis					
Necrotizing enterocolitis					
BW <1,500g					
BW <2,500g					
BW ≥ 2,500g					
Adherence to Treatment					
≥80% of vaginal progesterone					
≥50% of vaginal progesterone					
Adverse Effects of Interventions					
Vaginal Discharge					
Pain					
Itching					
Bleeding					
Other					

Appendix E: Sample Size Calculations

Completed using G*Power, version 3.1.9.6. for MacOS

- Based on 20% reduction of reported 60% preterm birth rate in twin gestations prior to 37 weeks
- Using Cox Proportional Hazard Regression
- α error = 0.05
- β error = 20%
- Power = 80%
- Calculated to be 97 in each arm

$$N_1 = \left\{ z_{1-\alpha/2} * \sqrt{\bar{p} * \bar{q} * (1 + \frac{1}{k})} + z_{1-\beta} * \sqrt{p_1 * q_1 + (\frac{p_2 * q_2}{k})} \right\}^2 / \Delta^2$$

$$q_1 = 1 - p_1$$

$$q_2 = 1 - p_2$$

$$\bar{p} = \frac{p_1 + kp_2}{1 + K}$$

$$\bar{q} = 1 - \bar{p}$$

$$N_1 = \left\{ 1.96 * \sqrt{0.5 * 0.5 * (1 + \frac{1}{1})} + 0.84 * \sqrt{0.6 * 0.4 + (\frac{0.4 * 0.6}{1})} \right\}^2 / 0.2^2$$

$$N_1 = 97$$

$$N_2 = K * N_1 = 97$$

- Accounting for maximum loss to follow-up = 25%
- Required sample size = 1.25*97 = 122

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