

Background

- ❖ Platelet play a crucial role in haemostasis through initial plug formation after vascular injury and aid in the propagation of the coagulation pathways.
- ❖ Platelet dysfunction has also been implicated in abnormal embryonic implantation and uteroplacental disorders such as recurrent foetal losses, pre-eclampsia and intra-uterine growth restriction (IUGR) - a theory supported by the beneficial use of aspirin in such cases.
- ❖ However, there is limited data on the changes of platelet function during normal pregnancy and puerperium. Available studies also have small sample sizes.
- ❖ Understanding the normal physiological changes is important to elucidate disease processes and aid in the development of appropriate management interventions.
- ❖ We study platelet aggregation during the three trimesters of uncomplicated pregnancy and postnatal period compared to healthy non-pregnant control group.

Method and Materials

- ❖ A prospective cross-sectional study. A total of 125 women recruited (25 women in each group of first, second and third trimesters of pregnancy, 25 postnatal and 25 non-pregnant control group).
- ❖ Case selection is based on specific inclusion and exclusion criteria (child-bearing age between 18 – 45 years, no significant medical history in particular absence of hypertensive disorders, chronic illnesses, bleeding or thrombotic disorders and not on medication known to affect platelet function (e.g. NSAIDS, antibiotics as per British Society for Haematology guidelines [Harrison et al, 2011]). Women with previous or current history of pre-eclampsia, gestational hypertension and small for gestational age babies (SGA or IUGR) were excluded.
- ❖ First trimester defined as viability to 14 weeks, second trimester 15 to 28 weeks, third trimester 29 week to delivery (42weeks) and postnatal after delivery to 42 days postnatal. Controls are non-pregnant women with no significant medical or drug history and not on hormonal contraception.
- ❖ A MCMDM-1VWD bleeding score questionnaire [Bowman et al, 2008] was done to rule out a bleeding history. Venous blood is obtained using a large-bore needle with minimal venous compression. Initial 5mls of blood is used for routine antenatal blood analysis or discarded. Subsequent 20mls blood sample is taken in citrated syringe bottles using slow withdrawing technique so not to agitate or activate the platelets.
- ❖ Platelet rich plasma (PRP) light transmission aggregometry (the gold standard for assessing platelet function [Harrison et al, 2011]) is then performed with PAP Model 8E using seven agonists (final concentrations of 2µM and 3µM ADP, 3µM epinephrine, 1µg/mL collagen, 1mmol/L Arachidonic acid (AA), 0.5mg/mL Ristocetin, 1.5mg/mL Ristocetin and 1µM synthetic thromboxane receptor analogue (U46619)

Results

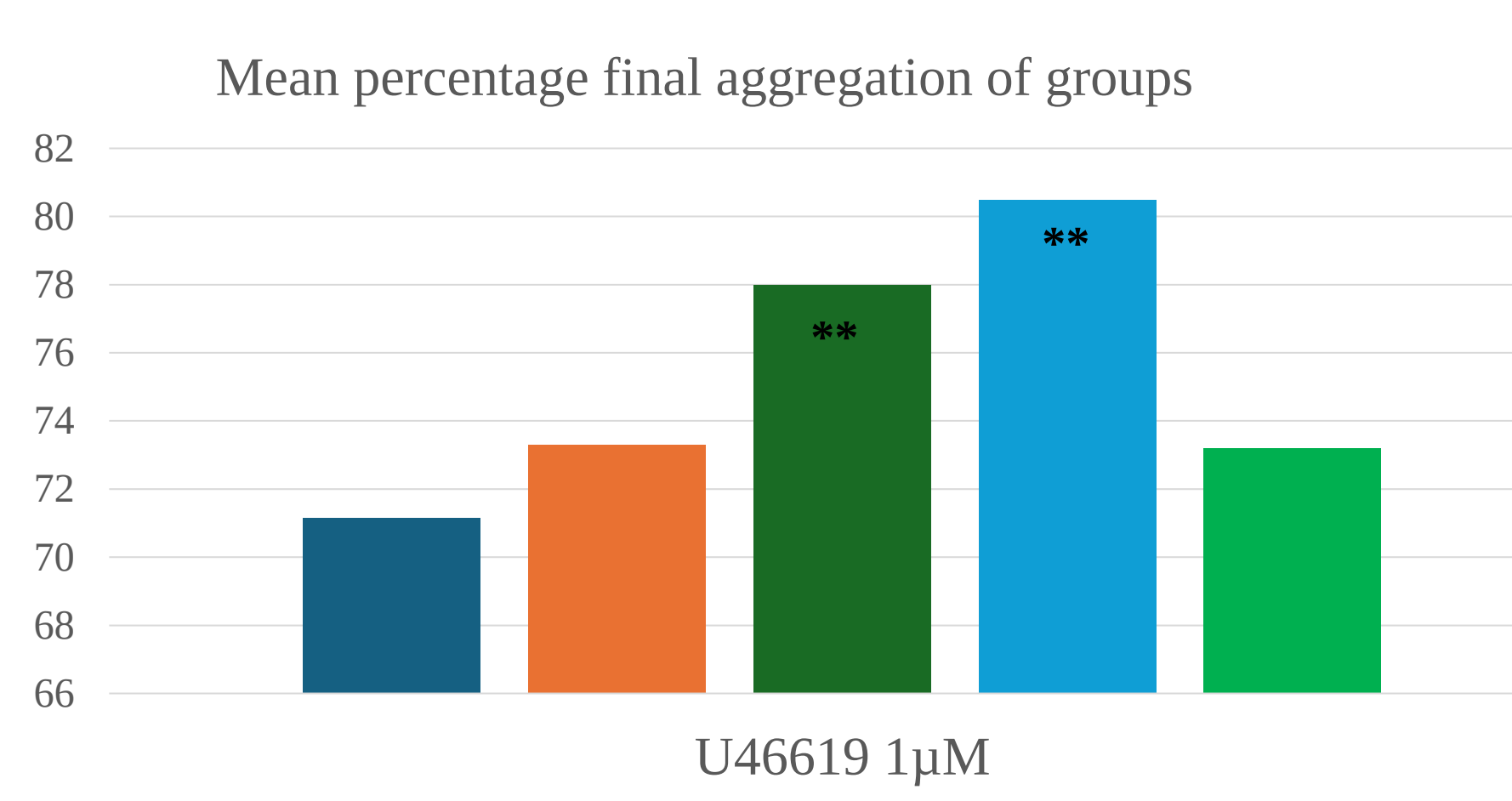
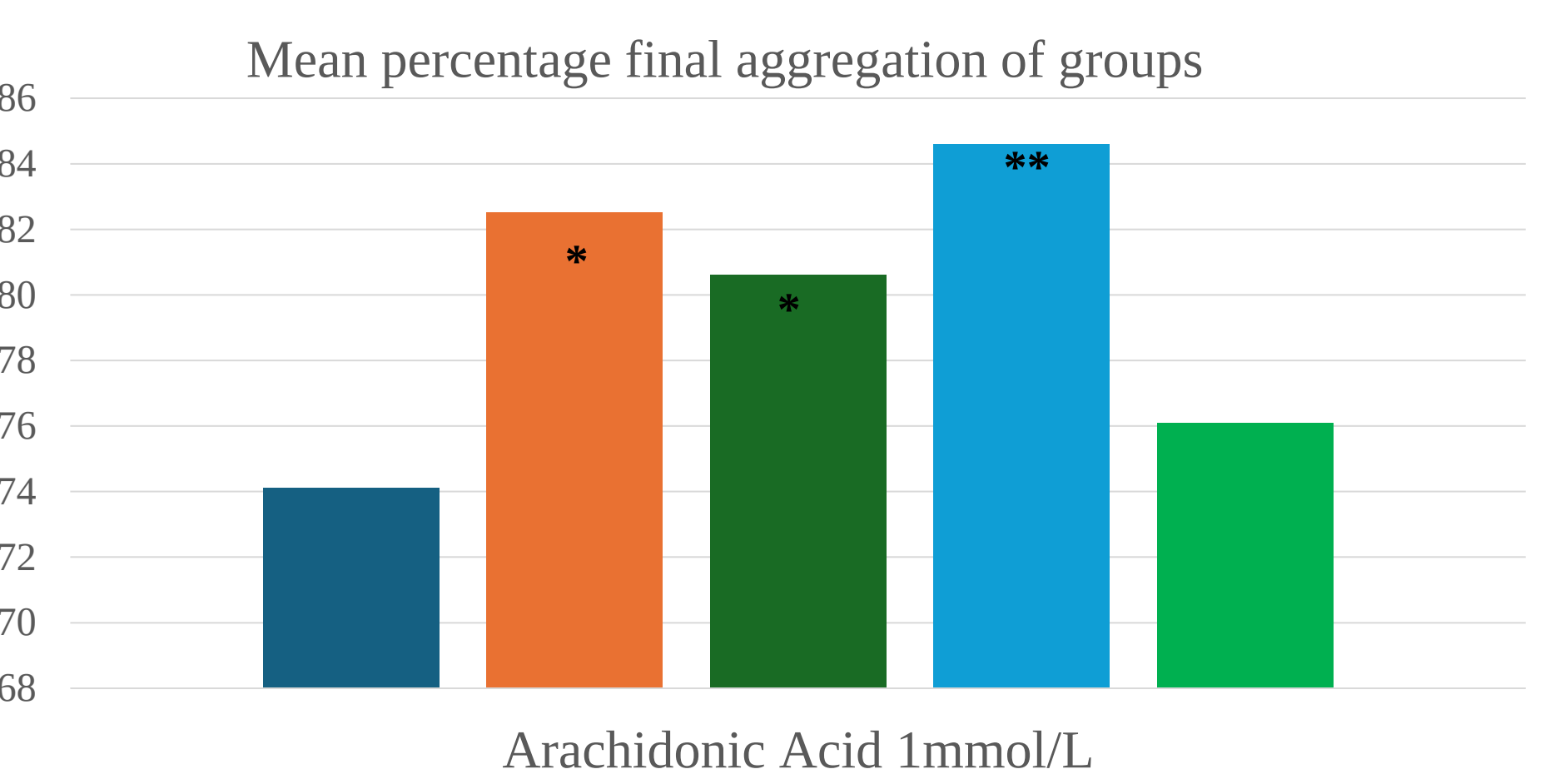
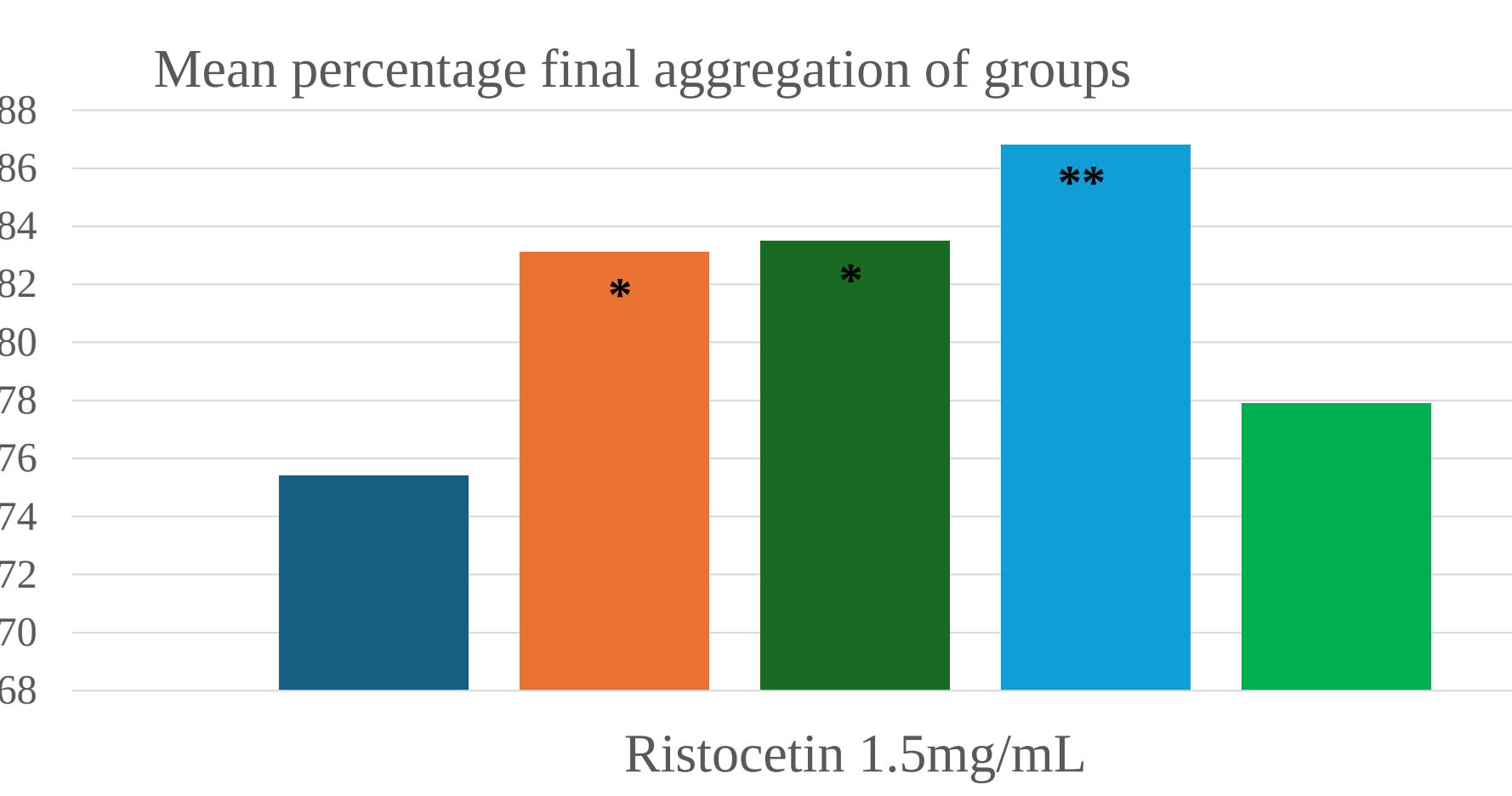
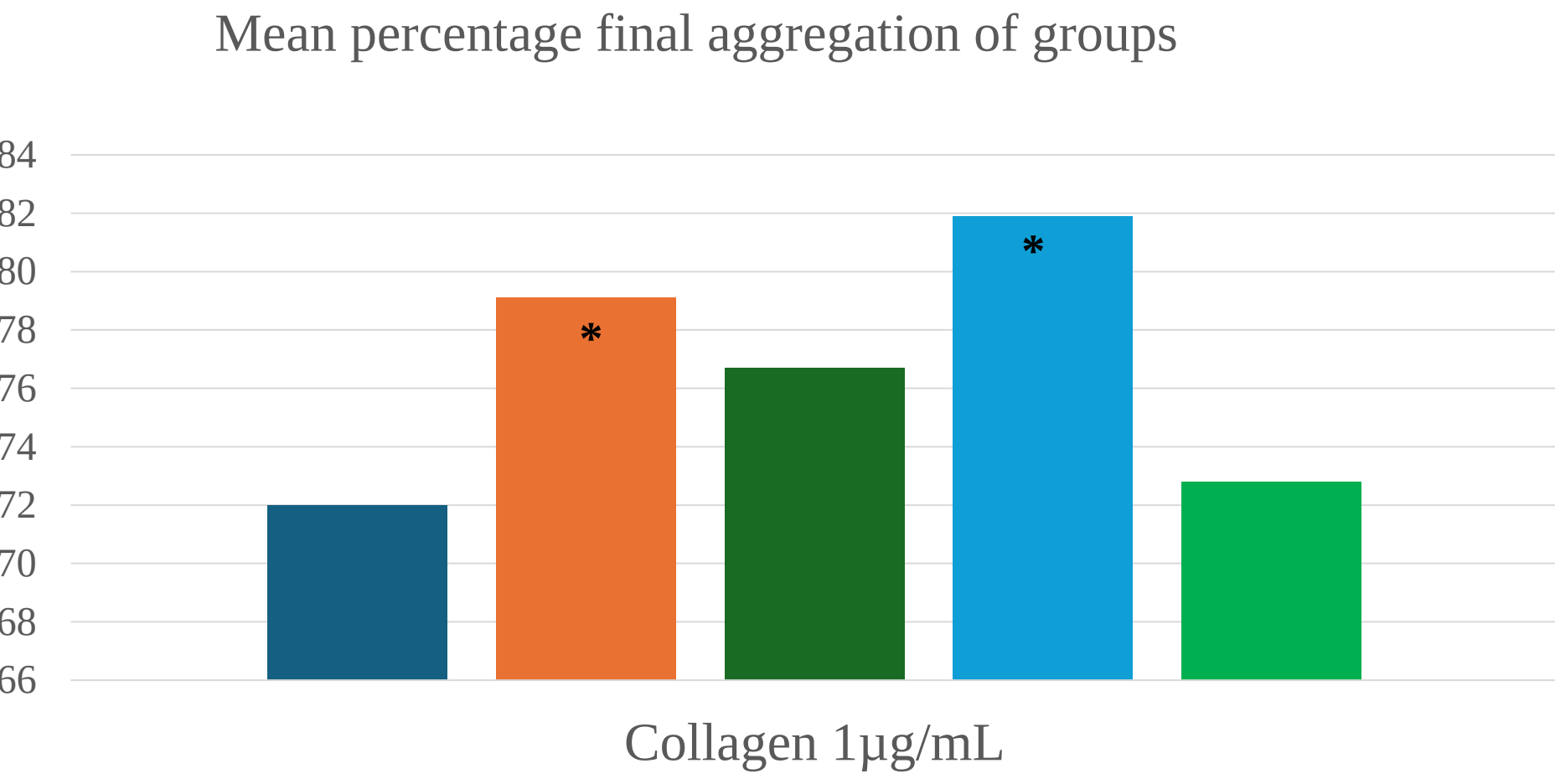
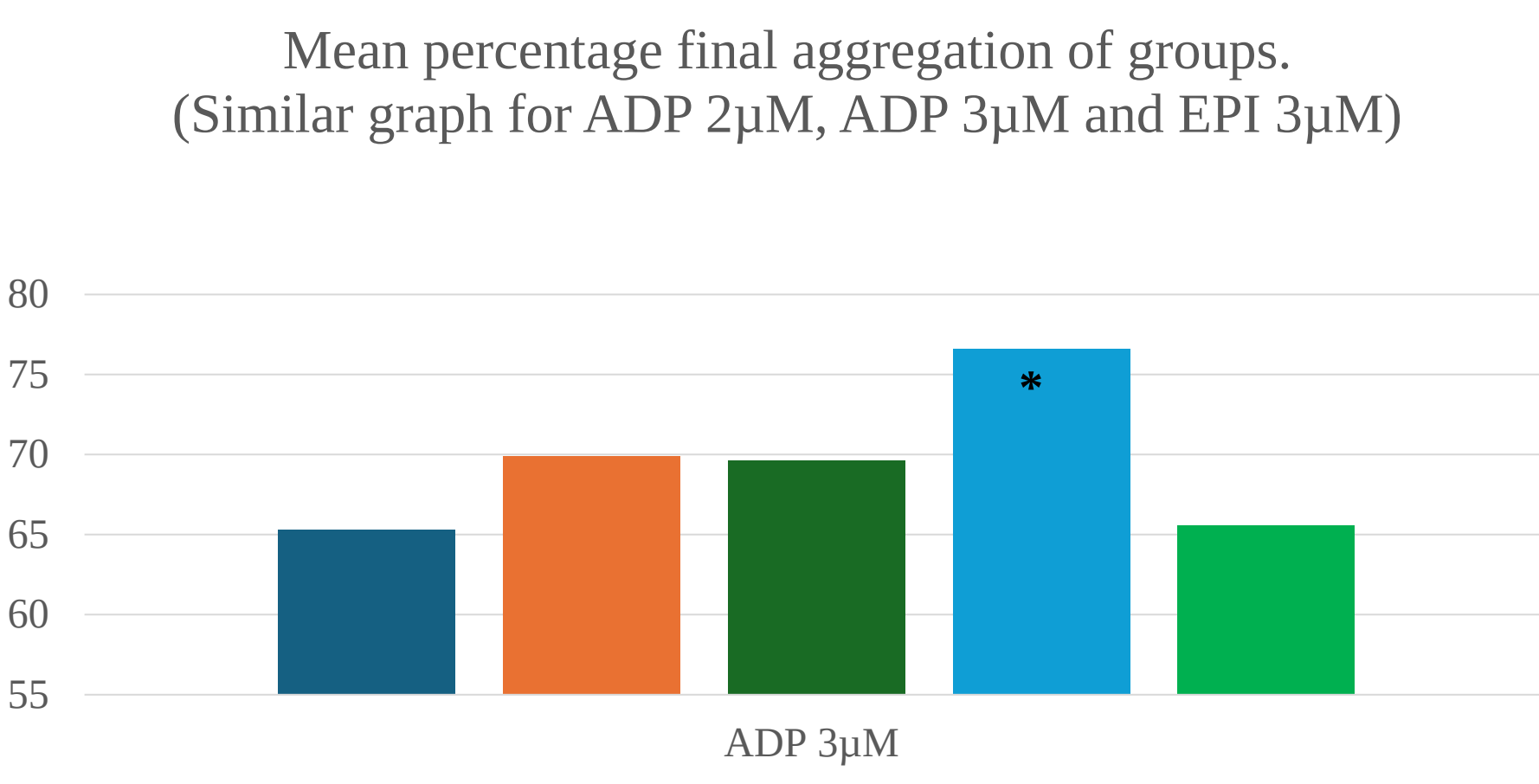
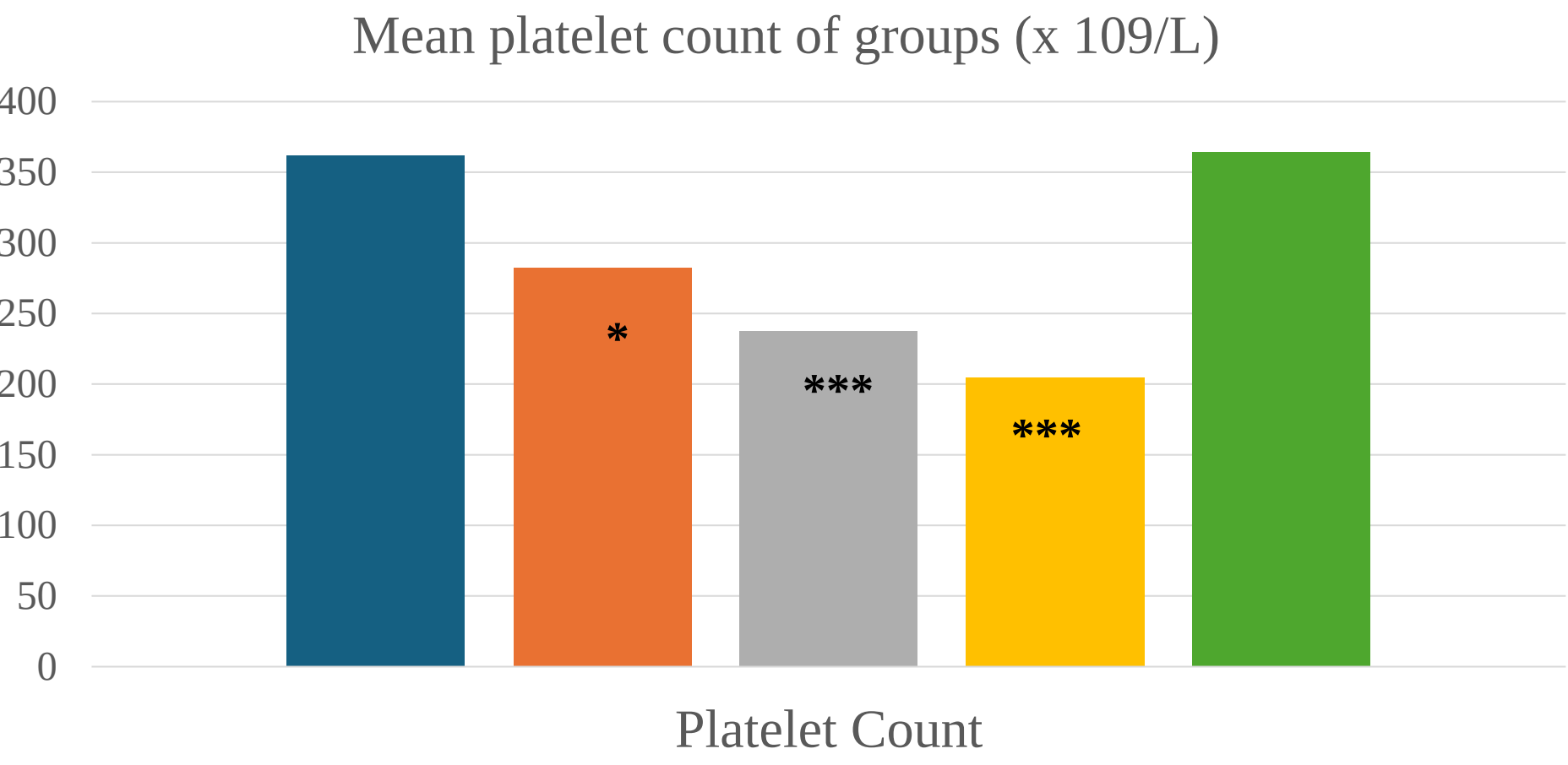
- ❖ Data from 125 women have been included in the results (25 first trimester, 25 second trimester, 25 third trimester, 25 postnatal women and 25 controls).
- ❖ The results of women taken in antenatal period of pregnancy who subsequently developed hypertensive disorders of pregnancy (PET and PIH) or had small babies (SGA/FGR) were excluded.
- ❖ Data analysis is done using STATA software. Independent T-test analysis is done to compare final platelet aggregation of each group to control. Mann-Witney U test is used for epinephrine 3µM as data distribution is skewed (non-parametric)

Table 1. Demographic characteristics between groups.					
	Control	First Trimester	Second Trimester	Third Trimester	Postnatal
	N = 25	N = 25	N = 25	N= 25	N = 25
Mean Age, years (SD)	33.3 (5.6)	30.8 (3.8)	31 (6.1)	29.8 (4.4)	31.7 (4.6)
Mean weight, kg (SD)	70.1 (15.3)	65.3 (15.8)	69.7 (14.4)	63.6 (13.9)	64.2 (9.6)
% Parous	43	46	52	42	100
Non-smoker status	100	100	100	100	100

Results expanded

- ❖ As previous shown in other research, platelet numbers reduces as pregnancy progresses in this study as well. In fact, the difference in platelet count is noticed in the first trimester group compared to controls and this difference widens as pregnancy progresses. This phenomenon is attributed to the haemodilution of pregnancy and platelet clumping seen in pregnancy.
- ❖ Final platelet aggregation to weak agonists (ADP and epinephrine) increases significantly in the third trimester compared to controls.
- ❖ Final platelet aggregation to strong agonists such as high dose ristocetin (1.5mg/mL) and arachidonic acid increases significantly from the first trimester through to the third trimester. It returns back to control levels in the puerperium. .
- ❖ Final platelet aggregation to U46619 increases significantly from the second trimester onwards.
- ❖ Although collagen show a statistically increased aggregation in the first trimester, this significant difference is lost in the second trimester and regained in the third trimester.
- ❖ Interestingly, platelet numbers and final aggregation to all agonists return to control levels in the late postnatal period (day 10 to 42)
- ❖ The demographic characteristics of all the groups appear similar overall except the control group appear to be older in age. Further statistical analysis is needed to evaluate its impact on results.

Keys for data interpretation
Coloured bars (always presented in sequence): **Controls**, **First Trimester**, **Second trimester**, **Third trimester**, **Postnatal**
P values calculated by independent t-test comparing a particular group to controls. * = **p< 0.05**; ** = **p < 0.001**; *** = **p< 0.0001**



Conclusions

- ❖ Overall, there is increased platelet aggregation to all the agonists as pregnancy advances. This is a desired physiological change to prevent haemorrhage around delivery. As increased platelet aggregation to some strong agonists starts in early pregnancy, this may be a protective mechanism to protect against life-threatening haemorrhage from early pregnancy.
- ❖ Interestingly, the comparable platelet aggregation of U46619 and collagen to controls in the first and second trimesters respectively may be a physiological modulation to aid in normal development of the fetal placenta around those time periods.
- ❖ The impact of this research is that it provides benchmark information regarding the changes in platelet function in uncomplicated pregnancy upon which future studies such as the predictability of peripartum bleeding using platelet function testing and the contribution of platelet dysfunction to the pathophysiology of hypertensive disorders of pregnancy and fetal growth restrictions can be studied.

References
Bowman M, Mundell G, Grabell J, Hopman WM, Rapson D, Lillicrap D, James P. Generation and validation of the Condensed MCMDM-1VWD Bleeding Questionnaire for von Willebrand disease. *J Thromb Haemost* 2008; 6: 2062–6.
Harrison P, Mackie I, Mumford A, Briggs C, Liesner R, Winter M, Machin S and British Committee for Standards in Haematology. Guidelines for the laboratory investigation of heritable disorders of platelet function. *British Journal of Haematology* 2011; 155: 30–44.