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# Effects of maternal parity on response of human myometrium to oxytocin and ergometrine in vitro

Gillian A. Ryan[a,b,*], Denis J. Crankshaw[a,b], John J. Morrison[a,b]

[a] Department of Obstetrics and Gynaecology, National University of Ireland Galway, Galway University Hospital, Ireland
[b] Lambe Institute for Translational Research, National University of Ireland, Galway, Ireland

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ABSTRACT

*Objective:* The aim of this study was to compare the effects of oxytocin and ergometrine on the intrinsic contractile parameters of human uterine smooth muscle at term between primiparous and multiparous women.

*Study design:* Myometrial biopsies were obtained from women undergoing planned caesarean section at term. The biopsies were dissected into eight uniform strips and mounted in tissue baths for isometric recording. The strips were challenged with increasing concentrations of oxytocin and ergometrine. Parameters of contractile activity, including mean contractile force (MCF) and maximum amplitude of contractions (MAMP) were recorded and analysed. Results were compared between primiparous (Group 1) and multiparous (Group 2) women.

*Results:* Myometrial biopsies were obtained from n = 11 donors (88 tissue strips), of which n = 5 were Group 1 and n = 6 were Group 2. In relation to oxytocin, the MAMP value observed was significantly greater in Group 2 than in Group 1 ($151 \pm 18$mN vs $67 \pm 14$mN, $P < 0.01$). Regarding ergometrine, the MCF response was greater in Group 2 samples ($24 \pm 10$ mN) than that in Group 1 ($18 \pm 2$mN) ($P < 0.05$).

*Conclusion:* Our findings highlight that women in a first pregnancy have a decreased response to both oxytocin and ergometrine in an in vitro setting when compared with women in a subsequent pregnancy, and this may have clinical implications regarding the management of postpartum haemorrhage in this cohort.



## Introduction

Obstetric haemorrhage, in particular primary postpartum haemorrhage (PPH), is a major cause of maternal morbidity and mortality in both the developed and developing world [1]. Recent estimates suggest that 27.1% of maternal deaths are due to major obstetric haemorrhage, with PPH accounting for two thirds of these cases [2]. As much as 26.7% of severe adverse maternal outcomes are also caused by PPH [3,4]. PPH is traditionally defined as blood loss >500 mL within the 24 h of delivery of the infant, and severe PPH, which most commonly occurs in the context of uterine atony, is blood loss of >1000 mL [1,3].

Oxytocin and ergometrine, either alone or in combination, are two of the most commonly used uterotonic agents in the management of PPH [1,5,6]. The Royal College of Obstetricians and Gynaecologists, London, recommends oxytocin as the agent of choice for PPH prophylaxis in the third stage of labour [7], as it has been shown to be superior to ergometrine alone [8]. For women at increased risk of PPH a combination of ergometrine–oxytocin is advised, in the absence of hypertension, as it has been shown to further reduce the risk of minor PPH (500–1000 ml) [7–9]. With regards to the treatment of PPH oxytocin is typically recommended as the first line agent and can be used in conjunction with other uterotonic agents and haemostatic procedures. There are minimal data available comparing oxytocin with other second line uterotonic agents. A Cochrane review on this topic concluded that an oxytocin infusion should be used first line in the treatment of PPH, though the evidence was limited, and there were no data available pertaining to oxytocin versus ergometrine use [1].

Apart from its status as the first line uterotonic agent used in the treatment of PPH, the effects of oxytocin have been well established in human myometrial studies in vitro [10–12]. We have previously demonstrated that oxytocin was the most potent uterotonic agent in vitro among a range of drugs used in the clinical treatment of PPH [13]. These findings have also been demonstrated by other groups [14]. Such scientific studies have supported a clear role for this drug as a first line agent in the treatment of primary

* Corresponding author at: Department of Obstetrics and Gynaecology, Clinical Science Institute, National University of Ireland Galway, Galway, Ireland.
*E-mail address:* gillian.ryan@nuigalway.ie (G.A. Ryan).

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PPH. What is not clear, nor previously investigated to our knowledge, is whether or not maternal parity exerts an effect on the potency of oxytocin, or other uterotonic agents, in human myometrium. The aim of this study was to compare the effects of oxytocin and ergometrine on the intrinsic contractile parameters of human uterine smooth muscle at term between primiparous and multiparous women. This study was presented at the Society of Maternal- Fetal Medicine (SMFM), Las Vegas, NV, USA 2019 [15].

## Materials and methods

### *Tissue sample collection*

Myometrial biopsies were obtained from women undergoing planned caesarean section (CS) performed in the third trimester of pregnancy, in the Department of Obstetrics and Gynaecology, Galway University Hospital, Galway, Ireland. This was done after obtaining written informed consent and with institutional review board approval, obtained from the Research Ethics Committee at Galway University Hospital (Ethics Reference Number C.A 1758 – Collection of Reproductive Tissue Samples at Time of Delivery, 13th July 2017). The samples were collected from the upper lip of the incision in the lower uterine segment as described previously [16–18]. Specimens were obtained prior to the administration of the routine bolus of oxytocin administered after delivery of the infant. All women recruited had planned CS procedures before the onset of labour. Exclusion criteria included the following: maternal age <18 years, previous vaginal delivery, previous labour (defined as cervical dilatation ≥4 cm), maternal blood borne infections, prior administration of exogenous oxytocin or prostaglandin agents, a diagnosis of gestational diabetes (GDM), and a perceived lack of patient understanding or capacity to consent. There were two groups of women recruited as follows: 1. Women undergoing CS in their first pregnancy; 2. Women undergoing CS who were in a second or subsequent pregnancy to term gestation.

Following collection of the samples in the operating theatre, they were placed in physiological salt solution (PSS) at 4 °C. The PSS contained the following ingredients: 4.7 mmol/L potassium chloride (KCl), 118 mmol/L sodium chloride, 1.2 mmol/L magnesium sulfate, 1.2 mmol/L calcium chloride, 1.2 mmol/L potassium sulfate, 25 mmol/L sodium bicarbonate, and 11 mmol/L glucose (Sigma-Aldrich, Dublin, Ireland). Within 2 h of collection specimens were transferred to the laboratory where they were transferred to fresh PSS and experiments were carried out within 15 h.

### *Tissue bath experiments*

Myometrial tissue biopsies from n = 11 women were dissected and prepared for tissue bath analysis. Decidua, serosa and scar tissue were removed, and each tissue sample was dissected into eight uniform strips of approximately 2 × 2 x 10 mm size, as previously described [10,16,19]. Only biopsies from which eight strips were obtained were included in the study, resulting in a total of 88 myometrial strips for analysis. Once prepared, the myometrial strips were mounted in tissue baths containing 20 mL of PSS, pH 7.4 at 37 4 °C [18,20] and were gassed continuously with a mixture of 95% oxygen/5% carbon dioxide.

The strips were then stretched to a resting tension of 20 mN and allowed 30-min equilibration time. Fresh PSS was then introduced, and the tension readjusted to 20 mN and equilibrated for a further 15-minute equilibration period. A period of spontaneous activity, without any further intervention, was recorded for 130 min. Strips were then challenged with potassium chloride (KCl, 60 mmol/l). After a 10-minute exposure period strips were then washed with fresh PSS and allowed a further 15-minute period of recovery.

### *Concentration-effect studies*

For the concentration effect studies the subsequent period of the experiment was divided into 5 intervals of 15-minute duration. Uterotonic agents were then added in concentrations that increased cumulatively by approximately one log unit every 15 min until five doses were applied. The starting doses (determined from dose finding experiments) were as follows: oxytocin (Sigma-Aldrich) 10 pmol/l and ergometrine (Hameln), 1.1 nmol/l. Each agent was tested on four strips of the same donor and agents were rotated through experiments in a Latin Square design.

### *Contractile activity measurements and data analysis*

Contractile activity was recorded using PowerLab/8SP recording unit writing to LabChart v7.37 software (AD instruments, Oxford, UK). Data were extracted using custom macros in LabChart v7.37 and the results exported to Microsoft Excel for analysis. Concentration effect curves were constructed to further assess results, using Graph Pad Prism software, as previously described [13]. The following parameters of contractile performance were measured for each 15-minute block of concentration-effect experiments: the maximum amplitude (MAMP) of contractions and the mean contractile force (MCF) above baseline. The -log$EC_{50}$ value obtained for each agent (as a measure of potency), in relation to the two contractile parameters, were also recorded. All forces were then normalized to the response to the KCl challenge.

All data from a given donor were pooled into a single concentration-effect curve. All values are expressed as the arithmetic mean +/- the standard error of the mean (s.e.m). The results were analysed for both clinical groups and compared. Statistical analysis across the groups was performed using a *t*-test (IBM SPSS Version 24), Mann Whitney U test, or Chi-square test as appropriate. Statistical significance was taken as a P value <0.05.

## Results

There were n = 11 donors and a total of 88 tissues strips for analysis of which n = 5 were Group 1 (40 strips) and n = 6 Group 2 (48 strips). The average maternal age, BMI and gestation at delivery are presented in Table 1. Women in Group 2 had a higher maternal parity and number of CS than those in Group 1. No other significant differences were observed in the demographics between the two groups. The median number of previous CS procedures in Group 2 was 1 (range 1–3). In Group 1 the indications for CS were as follows: placenta praevia n = 1, maternal/fetal indication n = 3, and fetal malpresentation n = 1. A previous CS constituted the indication for the index CS for all women in Group 2.

**Table 1**
Demographics of Group 1 and Group 2.

| | Group 1 (N = 5) | Group 2 (N = 6) | P Value |
|---|---|---|---|
| Age (Years) | 36.2 | 36.3 | *0.9* |
| BMI (kg/m [2]) | 28 | 29.3 | *0.7* |
| Gestation (weeks + days) | 38 + 5 | 38 + 6 | *0.5* |
| Parity<br>Median<br>Range | 0 | 1<br>(1-3) | *0.04* |
| Previous CS<br>Median<br>Range | 0 | 1<br>(1-3) | *0.04* |

Group 1 = Primiparous women. Group 2 = Multiparous women.

Representative recordings of myometrial contractility before and after addition of oxytocin and ergometrine are shown in Figs. 1A and 1B respectively. The MAMP and MCF values, and the logEC$_{50}$ results, are demonstrated for both uterotonic agents in Table 2. In relation to oxytocin, the MAMP value observed was significantly greater in Group 2 than in Group 1. There was no difference observed between MCF values elicited by oxytocin between both groups. For Ergometrine, there was a trend towards increased MAMP in Group 2, in comparison to Group 1(Table 2). Regarding MCF in response to ergometrine exposure, the mean MCF response to ergometrine was greater in Group 2 samples (23.9 ± 10.3 mN) than that in Group 1 (17.8 ± 2.0 mN) (P = 0.03; Table 1). In relation to potency for MAMP and MCF, the -log EC$^{50}$ values are shown in Table 2. There was no significant difference observed between these values. Similarly, there was no significant differences observed between the groups in their responses to the KCl challenge for either MAMP (P = 0.93) or MCF (P = 0.15).

The effects of each agent within the groups were compared and oxytocin elicited a significantly greater MCF than ergometrine in both Group 1 (P = 0.05) and Group 2 (P=<0.05). Regarding MAMP, the response elicited in Group 2 was greater for oxytocin than ergometrine (P < 0.05), and no difference was observed in Group 1.

**Table 2**
Mean Values of Contractile Parameters for Oxytocin and Ergometrine.

| | Group 1 (N = 5) | Group 2 (N = 6) | P Value |
|---|---|---|---|
| ***Oxytocin*** | 67 ± 14 | 151 ± 18 | *0.005* |
| ***MAMP (mN)*** | −8.8 ± 0.4 | −8.8 ± 0.3 | *0.9* |
| ***LogEC$_{50}$ (mN)*** | | | |
| ***MCF (mN)*** | 50 ± 7 | 61 ± 9 | *0.3* |
| ***LogEC$_{50}$*** | −8 ± 1 | −8 ±1. | *0.6* |
| ***Ergometrine*** | 47 ± 20 | 93 ± 11 | *0.07* |
| ***MAMP (mN)*** | −6.2 ± 0.5 | −6.7 ± 0.7 | *0.4* |
| ***LogEC$_{50}$*** | | | |
| ***MCF (mN)*** | 18 ± 2.0 | 24. ± 10 | *0.03* |
| ***LogEC$_{50}$*** | −7 ± 1 | −7.1 ± 0.5 | *0.7* |

Group 1 = Primiparous women. Group 2 = Multiparous women.
MAMP = Maximum amplitude; MCF = Mean contractile force.

## Discussion

This study outlines a comparative evaluation of the effects of oxytocin and ergometrine on uterine contractility in human myometrium in vitro, in biopsies obtained at term gestation. Knowledge regarding the pharmacological effects of these agents in human myometrium is important because of their clinical use in managing PPH [1,7,21,22]. This study was focused specifically on the issue of whether or not maternal parity exerted an effect on the sensitivity to and response elicited by these agents. The sensitivity to both agents in primiparous and multiparous women was the same (i.e. the -log EC$_{50}$ values were similar), but the response elicited was different in both groups. Oxytocin exposure resulted in a significantly greater maximum amplitude of contractions in multiparous women, while ergometrine exposure resulted in a greater mean contractile force, also in multiparous women. These findings indicate that the uterine contractile response to these two uterotonic agents may vary according to whether the woman is primiparous or multiparous. The findings are suggestive of an enhanced response to both agents in the multiparous woman in vitro.

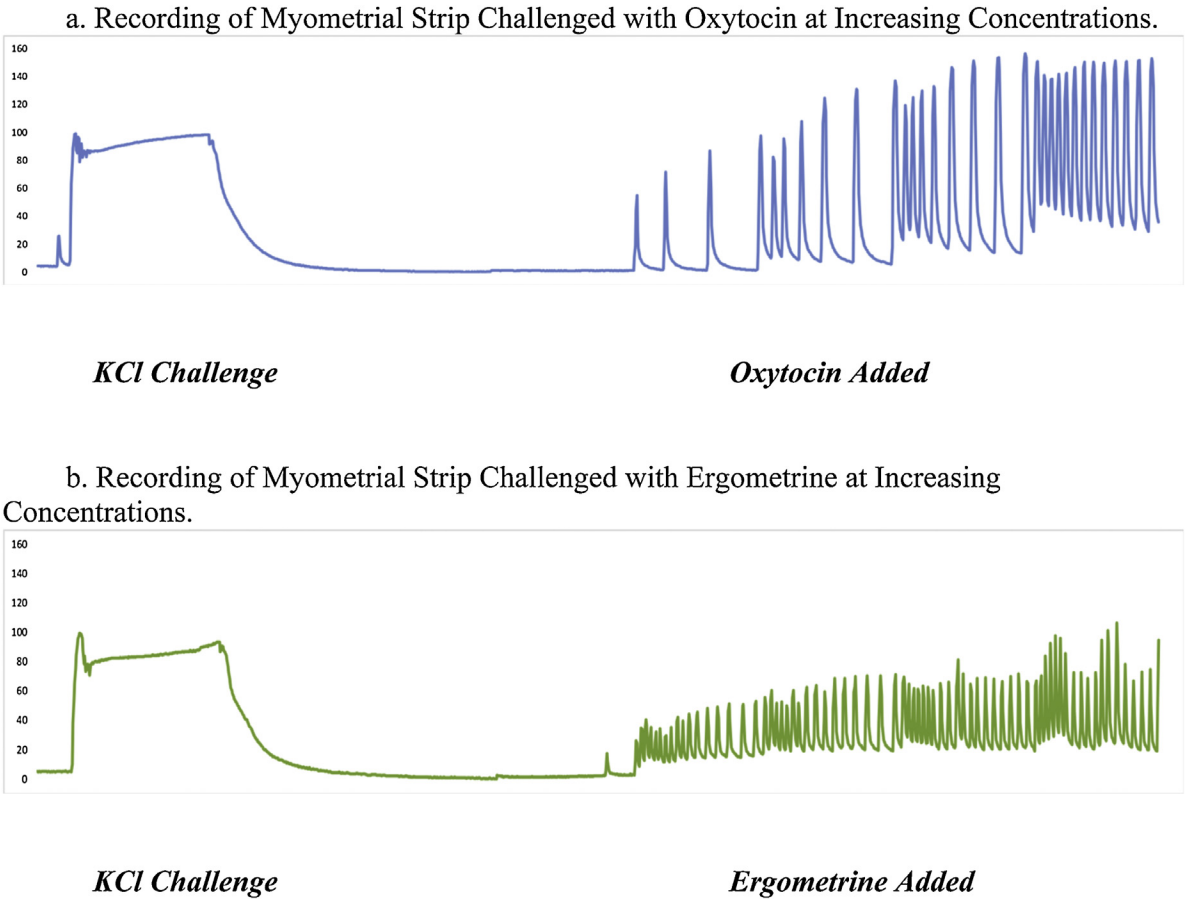


**Fig. 1.** Representative Recording of Myometrial Tissue Samples. Representative recording of myometrial strips challenged with increasing concentrations of oxytocin and ergometrine in Fig. 1a and b respectively. *KCl = Potassium Chloride.*

To our knowledge there are no other in vitro or clinical studies that have examined the effects of these two uterotonic agents in human uterine tissue in relation to parity. We have previously investigated the effects of parity on spontaneous uterine contractile activity in vitro and reported that no difference was observed between primiparous and multiparous women [18], and hence the effects observed here are most likely related to the agonist effect of the two agents. Speculation from in vitro studies regarding the in vivo situation is challenging, but our results suggest that the response to these agents may be greater in multiparous women.

There are however some limitations to the findings observed here. Firstly, all tissue samples were obtained before the onset of labour. It is challenging to obtain institutional review board approval to obtain a myometrial biopsy sample at the time of unplanned caesarean delivery. In addition, oxytocin administration is frequently in place for some time before such procedures are performed for failure to progress in labour and may exert an influence on the sensitivity and the results. The women recruited for this study had not received any prior uterotonic agents. Secondly, all samples were excised from the lower uterine segment and we are unable to state if the results apply in a similar way to upper segment uterine smooth muscle in pregnancy. Other workers have however reported that there were no functional differences between upper and lower segment myometrium in vitro [23]. There are also published data which have outlined that myometrial sampling poses no additional risks when performed at caesarean delivery, and obstetricians should be comfortable with obtaining full-thickness samples [24]. Thirdly, apart from parity, the women recruited to both groups differed in terms of number of prior caesarean deliveries performed. We have previously examined the effect of increasing numbers of CS on myometrial contractile parameters in an in vitro setting, and reported no differences observed [25]. The authors are therefore of the opinion that this was unlikely to have had an impact on the results. A further limitation of this study is extrapolating the results to the clinical environment, and in particular women in a labour setting. We acknowledge that uterine tissue from women who have a planned CS may respond differently to uterotonic agents than those after either a spontaneous or induced labour.

The findings from this study regarding the comparative effects of oxytocin and ergometrine on human myometrium in vitro concur with earlier reports [12–14]. Various in vitro studies have observed a superior contractile response of uterine muscle to oxytocin over ergometrine [12–14]. When we made these comparisons across our groups, we observed a similar result. The force and amplitude of contractions in Group 2 was significantly greater when exposed to oxytocin versus ergometrine. A similar finding of increased MCF when challenged with oxytocin versus ergometrine was observed in Group 1.

In conclusion this study has served to compare the effects of oxytocin and ergometrine on human uterine smooth muscle in the third trimester of pregnancy in relation to parity. Our findings highlight that women in a first pregnancy have a decreased response to both oxytocin and ergometrine in an in vitro setting when compared with women in a subsequent pregnancy, and this may have clinical implications regarding the management of PPH in this cohort.

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## Declaration of Competing Interest

The authors declare no conflict of interest.

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