THE UNIVERSITY of ADELAIDE

Management of sows and their piglets around the periparturient period: observing the effects on survival and performance of the litter

Sophia Aleksandra Ward

BSc. Honours (The University of Adelaide)

22/2/02

A thesis submitted in fulfillment of the requirements for the degree of Doctor of Philosophy (PhD)

CONTENTS

ABSTRACT

The periparturient period, defined as the period immediately before and after birth, is a challenging event for both sow and piglets. With piglet preweaning mortality being a significant issue in the pork industry, the aim of this thesis was to see whether a particular management strategy could be implemented to improve the survival or performance of the litter. The overarching hypothesis for this body of work was that manipulations during the key period would result in more robust piglets better able to survive to weaning. Chapter 1 identified that the periparturient phase is a period where sow and litter performance are intrinsically linked, so assistance to sow farrowing and/or lactation performances would be beneficial to piglets. This led to studies conducted with dexamethasone, and whether this potent anti-inflammatory steroid could relieve sows of discomfort and assist in farrowing and early lactation performance. Administering dexamethasone to sows on the day before farrowing reduced piglet birthweights ($P < 0.001$), weaning weights ($P < 0.001$), plasma total protein concentrations ($P = 0.001$), and colostrum intake of piglets ($P = 0.006$). When dexamethasone was given to primiparous sows on the morning of farrowing, no adverse effects were evident for birth or weaning weights, plasma protein or colostrum intake ($P > 0.05$), and improvements were seen to the daily weight gain of piglets to weaning ($P = 0.01$; Chapter 2). When investigated further, the administration of dexamethasone on the day of farrowing did not influence the number of pain related behaviours, posture changes or time spent in lateral recumbency for primiparous and parity one sows ($P > 0.05$). Considering dexamethasone is a glucocorticoid, we also investigated whether this maternal treatment was influencing fetal maturity, specifically gut macromolecule permeability in low birthweight piglets (Chapter 4). The maternal treatment with dexamethasone did not affect the rate of gut permeation pre-closure ($P > 0.05$), opening up further queries as to how dexamethasone was able to improve daily weight gain of piglets in Chapter 2.

A theme running through the conducted studies was that having control over the farrowing process may be critical to effectiveness of a tested management technique or treatment. Maternal dexamethasone may improve piglet performance, but the chances of unfavorable side effects with improper timing makes these advantages difficult to realise To have greater control over the timing of farrowing onset, a drug delivery

system was formulated to trigger induction of parturition in sows (Chapter 5). The deposition of the novel drug delivery system into the vagina successfully resulted in farrowing onset with similar efficacy as sows induced via localized injection (current practice). The non-injection formulation of this novel inducing agent for sows has the potential to provide farmers with better control of the farrowing process, test management strategies for improving performance and ensure piglets at-risk are properly managed, in addition to moving away from injectable medications.

"Anyone who wants to be sure of keeping animal welfare on the political agenda in the future will need more coherent arguments than are currently used. That means more science."

Marian S. Dawkins, Oxford University, 2012

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint award of this degree. The author acknowledges that copyright of published works contained within the thesis resides with the copyright holder(s) of those works. I give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library Search and also through web search engines, unless permission has been granted by the University to restrict access for a period of time. I acknowledge the support I have received for my research through the provision of an Australian Government Research Training Program Scholarship.

— ————

Sophia Ward

Date: 22/02/2022

ACKNOWLEDGEMENTS

First and foremost, I would like to thank my primary supervisor: Associate Professor Roy Kirkwood. Undertaking this PhD has been such an incredible experience and it would have not been possible without your constant and very reliable support. Thank you for your outstanding guidance throughout my candidature and for your always patient and kind demeanor.

I would also like to express my sincere gratitude to my secondary supervisors, Dr. Kate Plush and Professor Sanjay Garg. Kate, thank you for spending countless afternoons with me, walking through the correct statistical application or response to a journal edit. Your own contributions to improving welfare in the pork industry has and will continue to be an inspiration. To Professor Garg, being part of your group at UniSA was an honor and I am so thankful for the opportunity. Learning about pharmaceutical applications may have been challenging at times, but you and Dr. May always helped me through with overwhelming support. My time at the Pharmaceutical Innovation and Development Group was one I will never forget, thank you for taking me in like family and allowing me to develop friends I will cherish for life.

To Tanya Nowland, Serena Barnes, Bryony Tucker, Sarah James, and Lisa McKenny, thank you so much for your support during my projects at the Piggery and being such fun and lovely people to work with. Thank you to Simon, Karina, Alex and all of the staff at the Roseworthy piggery for allowing me to conduct my projects with ease. I would also like to express my sincere gratitude to every veterinary and animal science student who gave up their time to help me during my trial periods.

Alongside academic support, I want to express my deepest thanks to the mental support I have received along the way. From my supervisors, workmates and of course my beautiful family. Mum and Dad, thank you for always believing in me and supporting me every step of this journey. Thank you to my sisters Sadie and Arabella for always being a supportive shoulder to listen to my troubles and help me celebrate my successes. And to my partner Damien, thank you for coming up to the piggery with me during my night trials after my helper got sick. This may be a small example of your kindness, but it means everything to me.

Finally, I am very grateful to the University of Adelaide and Australian Pork Limited for funding my stipend and allowing me to conduct this research

NOMENCLATURE

11-b-HSD-2	11-b-hydroxysteroid Dehydrogenase Type 2
ACTH	Adrenocorticotropic Hormone
ATP	Adenosine Triphosphate
BORIS	Behavioral Observation Research Interactive Software
BW	Bodyweight
Ca(OH)2	Calcium Hydroxide
CCTV	Closed Circuit Television
CI	Colostrum intake
Cox I/II	Cyclooxygenase enzymes
CR	Controlled release
Dex	Dexamethasone
Dex24	Dexamethasone administered 24 hours after induction (day 115 of gestation)
DexInd	Dexamethasone administered at the same time as induction (7am at day 114 of gestation)
DexInj	Dexamethasone was administered to sows via intramuscular injection
DexTop	Dexamethasone was administered topically into sow vagina
DexTwice	Dexamethasone administered at induction and 24 hours after induction (day 114 and day 115 of gestation)
Fig	Figure
FITC-D	Fluorescein isothiocyanate dextran
g	Grams
GCRs	Glucocorticoid receptors
GCs	Glucocorticoids

GH	Growth Hormone
h	Hour
HPA	Hypothalamic Pituitary Adrenal Axis
HPLC	High Performance Liquid Chromatography
HPMC	Hydroxypropyl Methylcellulose
HSP90	Heat shock protein 90
IBM SPSS	Software package used for statistical analysis
IGF-1	Insulin-like Growth Factor
IgG	Immunoglobulin G
In-vitro	Tested in a controlled environment with tissue extract
In-vivo	Tested on a living organism
IUGR	Intrauterine Growth Restriction
kg	Kilogram
kgF	Kilogram force
kJ	Kilojoules
kJ/kg BW	Kilojoules per Kilogram Bodyweight
KOH	Potassium Hydroxide
LBW	Low birthweight
mg	Milligrams
min	Minute
mL	Milliliters
mRNA	Messenger Ribonucleic Acid
n	Number of animals
NaCl	Sodium Chloride
NBW	Normal birthweight
nm	Nanometer
NSAID	Non-Steroidal Anti Inflammatory
NVDDS	Novel vaginal drug delivery system

P0	Gilt (Parity zero)
P1	Sow in first parity
P1-P5	Sows in their first parity to sows in their fifth parity
PGF2α	Prostaglandin F2alpha
Rpm	Reps per minute
RR	Rapid release
SAID	Steroidal Anti Inflammatory
SPVF	Simulated porcine vaginal fluid
TNF-α	Tumour Necrosis Factor alpha
μg	Micrograms

LIST OF TABLES

Chapter 5: Development of a novel drug delivery system for sows to control timing of piglet delivery and improve farrowing supervision

LIST OF FIGURES

List of publications included in thesis

Chapter 1.

Ward, S. A., Kirkwood, R. N., & Plush, K. J. (2020). Are larger litters a concern for piglet survival or an effectively manageable trait? *Animals*, *10*(2), 309.

Chapter 2.

Ward, S. A., Kirkwood, R. N., & Plush, K. J. (2020). Administering dexamethasone to prepartum sows: Effects on sow and piglet performance. *Livestock Science*, *239*, 104171.

Chapter 3.

Ward, S. A., Kirkwood, R. N., Song, Y., Garg, S., & Plush, K. J. (2022). Effect of Dexamethasone and Route of Administration on Sow Farrowing Behaviours, Piglet Delivery and Litter Performance. Animals, 12(7), 847.

Chapter 5.

Ward, S. A., Kirkwood, R. N., Plush, K. J., Abdella, S., Song, Y & Garg. S. (2022). Development of a novel vaginal drug delivery system to control time of farrowing and allow supervision of piglet delivery. *Pharmaceutics,* 14, 340.

List of other publications written during candidature

Ward, S. A., Kirkwood, R. N., & Plush, K. L. (2019). Effects of oxytocin and carbetocin on farrowing performance. *Animal reproduction science*, *205*, 88-93.

Song, Y.; Abdella. S.; Kim, S.; Youssef, S.; Abid. F.; **Ward. S.;** Garg, S. pH and its application in targeted drug delivery systems. (2021) *J. Control Release.* ***** pending publication *****

Conference abstracts written during publication

Australasian Pig Science Association 17th Biennial Conference

Ward, S. A., Kirkwood, R. N., & Plush, K. J. (2019). Effect of glucocorticoid administered to sows at farrowing on piglet performance.

Australian Association of Animal Sciences (AAAS) Conference

Ward, S. A., Kirkwood, R. N., & Plush, K. J. (2022). Administering glucocorticoids to primiparous sows: effects on macromolecule uptake of low birthweight piglets.

Chapter 1.1: Introduction

General Background

As the global human population increases, total consumption of meat and animal products has risen considerably [1-6]. The pork industry has evolved with this growing demand by placing importance on increased productivity and output volumes of the breeding sow [7-9]. Through genetic selection it is now not uncommon for the modern sow to give birth to 15-20 piglets in a single litter [10]. Although advancements to productivity are testament to scientific prowess and farming methods, the survival of piglets in modern production are still subject to a sow's ability and willingness to manage the litter during and immediately after farrowing. In intensive production, it is common for sows in farrowing crates to be restricted from instinctual nesting behaviours in the hours leading up to parturition [12,13]. This can lead to the sow experiencing frustration, stress and/or aggression as they lead into the expulsion phase of farrowing [11-14]. After giving birth to piglets, the sow is then expected to nurse the entire litter unless fostering techniques are applied. During this time, known as the periparturient period, the sow and litter experience significant changes as the piglets must adapt to the challenges of extrauterine life with complete reliance on maternal lactation output. This is also the time when piglets are at the greatest risk of preweaning mortality [15] which is a major issue in the pork industry [16-19]. Preweaning piglet mortality is a significant issue and one that will continue to slow down productivity, waste resources and cost lives of piglets if not properly addressed. It is therefore important to review how the sow and litter can be assisted during the periparturient period to maximize survival and produce more robust piglets that are better able to survive to and beyond weaning.

The work outlined in this thesis was conducted to see whether a certain strategy, treatment or management program could be implemented during the periparturient period to maximize piglet survival and/or growth to weaning. This was investigated by considering possible issues that can arise with larger litters, conducting experiments that focused on the general sow, at-risk primiparous sows, then on at-risk piglets. The pursuit of an effective management strategy led to the development of a non-injectable induction agent that facilitated farrowing onset during working hours when piggery staff could intervene if needed. It is hoped this delivery system could be used on farms

to enable workers to implement a range of effective management strategies to assist the sow and/or litter.

References

1. Alexandratos, N.; Bruinsma, J. World agriculture towards 2030/2050: the 2012 revision. **2012**.
2. Henchion, M.; Moloney, A.; Hyland, J.; Zimmermann, J.; McCarthy, S. Trends for meat, milk and egg consumption for the next decades and the role played by livestock systems in the global production of proteins. *Animal.* **2021**, 100287.
3. M van Dijk, M.; Morley, T.; Rau, M.L.; Saghai, Y. Systematic review and meta-analysis of global food security projections to 2050. **2021**.
4. Anisovich, A.; Tsarik, P. Agricultural trends of the global economy. **2021**.
5. Whitnall, T.; Pitts, N. Global trends in meat consumption. *Agri. Commod.* **2019**, *9*, 96-99.
6. Szűcs, I.; Vida, V; Commerce. Global tendencies in pork meat-production, trade and consumption. *Appl. Agric. Res.* **2017**, *11*, 105-111.
7. Kim, G.W. Changes of Reproductive Performances in Breeding Sow Farms. *Resour. Sci. Res.* **2019**, *1*, 51-57.
8. Andersson, E. Impact of litter size on sow health and welfare. **2018**.
9. Hartung, J. A short history of livestock production. In *Livestock housing: Modern management to ensure optimal health and welfare of farm animals*; Wageningen Academic Publishers: 2013; pp. 81-146.
10. Kobek-Kjeldager, C.; Moustsen, V.A.; Theil, P.K.; Pedersen, L.J. Effect of litter size, milk replacer and housing on behaviour and welfare related to sibling competition in litters from hyper-prolific sows. *Appl. Anim. Behav. Sci.* **2020**, *230*, 105032.
11. Marchant-Forde, J.N. Merchant-Forde, R. M. *The welfare of pigs*; Springer Science & Business Media: 2009; Volume 7.
12. Weber, R. Alternative housing systems for farrowing and lactating sows. *Publ. Euro. Assoc. Anim. Prod.* **2000**, *102*, 109-116.
13. Baxter, E.M.; Jarvis, S.; Sherwood, L.; Robson, S.K.; Ormandy, E.; Farish, M.; Smurthwaite, K.M.; Roehe, R.; Lawrence, A.B.; Edwards, S.A. Indicators of piglet survival in an outdoor farrowing system. *Liv. Sci.* **2009**, *124*, 266-276.
14 Baxter, E.M.; Andersen, I.L.; Edwards, S.A. Sow welfare in the farrowing crate and alternatives. In *Advances in pig welfare*; Elsevier: 2018; pp. 27-72.
15. Alonso-Spilsbury, M.; Ramirez-Necoechea, R., Gonzalez-Lozano M.; Mota-Rojas, D., Trujilo-Ortega, M.E. Piglet survival in Early Lactation: A review. *J. Anim. Vet. Adv.* **2007**, *6*, 76-86.
16. Oliviero, C.; Junnikkala, S.; Peltoniemi, O. The challenge of large litters on the immune system of the sow and the piglets. *Reprod. Domest. Anim.* **2019**, *54*, 12-21.
17. Rutherford, K.; Baxter, E.; D'eath, R.; Turner, S.; Arnott, G.; Roehe, R.; Ask, B.; Sandøe, P.; Moustsen, V.; Thorup, F. Edwards, S.A. Lawrence, A.B. The welfare implications of large litter size in the domestic pig I: biological factors. *Anim. Welf.* **2013**, *22*, 199-218.
18. Koketsu, Y.; Iida, R.; Piñeiro, C. A 10-year trend in piglet pre-weaning mortality in breeding herds associated with sow herd size and number of piglets born alive. *Porc. Health Manag.* **2021**, *7*, 1-8.
19. Bunter, K.L. Managing consequences of increasing litter size: a genetic perspective. In Proceedings of the Manipulating Pig Production XII, Proceedings of the Twelth Biennaial Conference of the Australasian Pig Science Association, Australian Pig Science Association, Brisbane, Australia, 2009; pp. 149-156.

Thesis format

Chapter 1 provides a general background on the thesis topic and reasoning for project aims. It also includes a published literature review titled 'Are large litters a concern for piglet survival or an effectively manageable trait?' that provides scope of the issues that can arise during farrowing and strategies that have been investigated to assist sow and/or piglets during this time.

Chapter 2 describes an investigation of the use of a steroidal anti-inflammatory compound on farrowing performance and piglet survival, entitled 'Administering dexamethasone to prepartum sows: effects on sow and piglet performance'. This published journal article examined whether provision of dexamethasone, a potent anti-inflammatory compound, prior to farrowing would improve sow performance during parturition and early lactation.

Chapter 3 took key findings from the previous study and, in efforts to decipher how dexamethasone improved daily gain in litters of primiparous sows, focused on behaviour and posture changes, titled Effect of dexamethasone and route of administration on sow farrowing behaviours, piglet delivery and litter performance'. Alternative routes for dexamethasone administration were also explored by assessing permiation of the drug through porcine vaginal mucosa in-vitro.

After finding dexamethasone had little effect on the behaviour of primiparous sows during and the first 24 h after parturition, **Chapter 4** looks at whether dexamethasone could influence post-partum gut maturation in low birthweight piglets. This paper is titled 'Administering glucocorticoids to primiparous sows: effects on macromolecule uptake by low birthweight piglets'.

With our previous studies requiring sows to be induced to farrow to control timing of management strategies, **Chapter 5** presents the development of a non-injection protocol for farrowing induction. This study was undertaken as a visiting student to the Pharmaceutical Innovation and Development Group at the University of South

Australia. This paper is titled 'Development of a novel vaginal drug delivery system to control time of farrowing and allow supervision of piglet delivery'.

Chapter 6 summarizes the main findings from the previous chapters, explores the possible implications of this research and presents future opportunities for investigation.

This thesis is presented in a thesis-by publication format. Each chapter contains a journal article that has either been published or is currently undergoing peer review.

Project aims

The aim of this thesis was to see whether management of the sow and litter around the periparturient period could improve piglet survival and performance to weaning. If certain treatments or management strategies could improve survival of at-risk piglets in a litter and/or growth of piglets to weaning, there would be merit to breeding large litters for improved production efficiency.

In this investigation it was hypothesised that a particular management strategy could be implemented to improve survival of the litter. It was also predicted that, during the periparturient phase, improvements in sow farrowing and/or lactation performance will directly improve performance of the litter.

To test these predictions, the following project aims were proposed:

1) Determine whether administering dexamethasone during the immediate prepartum period influences sow and piglet traits important for piglet survival;
2) Find the most appropriate timing for dexamethasone administration;
3) Control the timing of farrowing in order to apply dexamethasone appropriately.

Project aims 1 and 2 were developed from research collated in from the literature review (Chapter 1.2), and project aim 3 was developed as a result of Chapters 1 – 4.

1

Chapter 1.2: Literature Review

Statement of Authorship

Title of Paper	Are larger litters a concern for piglet survival or an effectively manageable trait?
Publication Status	[X] Published [ ] Accepted for publication [ ] Submitted for publication [ ] Unpublished and Unsubmitted work written in manuscript style
Publication Details	Ward, S. A., Kirkwood, R. N., & Plush, K. J. (2020). Are larger litters a concern for piglet survival or an effectively manageable trait? Animals, 10(2), 309. DOI: https://doi.org/10.3390/ani10020309

Principal Author

Name of Principal Author (Candidate)	Sophia Ward		
Contribution to the Paper	Investigation of the literature, conception of the theme and writing and drafting of the review		
Overall percentage (%)	80 %		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	22/02/2022

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Roy Kirkwood		
Contribution to the Paper	Conception of the theme and drafting of the manuscript (10%)		
Signature		Date	22/02/2022
Name of Co-Author	Kate Plush		
Contribution to the Paper	Drafting and evaluation of manuscript (10%)		
Signature		Date	22/02/2022

animals

MDPI

Literature Review

Are Larger Litters a Concern for Piglet Survival or An Effectively Manageable Trait?

Sophia A. Ward[1]*, Roy N. Kirkwood[1], Kate J. Plush[2]

[1] School of Animal and Veterinary Sciences, University of Adelaide, SA, Australia
[2] Sunpork Group, Shea-Oak Log, SA 5371, Australia

Received: 23 October 2019; Accepted: 12 February 2020; Published: 17 February 2020

Simple Summary: In the swine industry, sows are selectively bred for larger litters so, theoretically, more pigs can be sold per year. As producers continue to increase the number of piglets born in a litter, it is necessary to review problems that can arise in larger litters, and whether these issues can be effectively managed and/or require pharmacological intervention. Additionally, this review will reflect on whether selecting sows for larger litter sizes is an ethical concern, regardless of how effectively it can be managed.

Abstract: As sows continue to be selected for greater prolificacy, it is important to review problems that arise in larger litters, and whether these issues can be appropriately managed. Although a proportion of piglets in larger litters can be born underweight, proper supervision around farrowing and adequate colostrum intake has the potential to improve the survival of low birthweight piglets and their ongoing growth to weaning. As larger litters can impart greater stress and discomfort on sows, implementing a low-stress environment leading up to parturition may improve sow performance and subsequent survival of piglets. Additionally, treating sows with anti-inflammatory compounds, either dietary or pharmacologically, shows some promise for alleviating sow discomfort and improving piglet survival in larger litters. Understanding that selecting sows for larger litters not only affects piglet survival but the well-being of the sow, the decision to continue selecting for larger litters, regardless of management strategies, remains a topic of ethical concern

Keywords: farrowing; management piglets; pre-weaning; mortality;

1. Introduction

As advances in genetics [1–3], reproductive management [4], and nutrition [5,6] continue to increase the number of piglets a sow can produce per litter [7,8], it is important to consider the issues that are associated with larger litters, particularly surrounding piglets and pre-weaning survival. Issues surrounding large litters include the effects of intrauterine crowding and so birth weight variation, piglet hypoxia during delivery and litter-mate competition post-partum. Potential management strategies for improving survival in large litters will be examined, including effective piglet fostering techniques, altering sow environments to reduce stress and the provision of anti-inflammatory compounds, both medicinal and dietary, to alleviate discomfort and improve performance. For this review, litter size is defined as all piglets born in a litter, born dead and alive, that would have contributed to intrauterine crowding during development. In turn, studies with varied interpretations of litter size will also be mentioned. The aim of this review is to identify factors that contribute to high piglet mortality in large litters, and by doing so suggest interventions that reduce the risk of piglet death.

2. Issues surrounding larger litters

2.1. Intra-uterine crowding and its impact on piglet development

Although sows have the capacity to conceive larger litters, uterine space and blood supply are limited resources [9-11]. On average, pregnancy is initiated in sows with the presence of around 15-20 viable embryos [11]. In an average litter, 9-13 of these embryos will eventually develop into live born piglets [12] but litters greater than 16 piglets are no longer uncommon in commercial production [13,14]. In larger litters, the uterus of a sow is crowded with embryos. When intrauterine crowding occurs, embryos first to implant can physically restrict the development of later attaching embryos, and this embryonic competition increases with every successful embryonic attachment [15]. Additionally, once the uterus has surpassed normal limits of uterine space, every additional littermate is associated with a reduction in individual fetal growth [16,17] In turn, larger litters are strongly correlated with a proportion of piglets born underweight (<1.0kg) [8,10]. Observing the performance of 965 litters, Quiniou et al. [7] found larger litters had a 33 gram decrease in mean birthweight average over 'normal' litters at 11

pigs. As low birth weight piglets have a larger surface area to volume ratio, they are more susceptible to weakness, hypothermia, and hypoglycemia within the first 24 hours of life [18]. Thus, low birthweight pigs have an increased risk of pre-weaning mortality compared to normal weight pigs [19-21]

Quiniou et al. [7] found that selection for larger litters not only reduced the mean birth weight in the litter, but also the uniformity of birth weight between littermates. Due to embryonic competition, pigs at the beginning of the order are usually heavier than subsequent littermates [19]. Variability within a litter makes it more difficult for low-birth-weight pigs to compete for a teat and ingest an adequate amount of colostrum. In addition, larger pigs compete indirectly with smaller pigs by draining and having more milk directed to their respective teats [22]. This indirect competition between littermates may explain why differences in bodyweight at birth are often maintained and even exacerbated throughout lactation [23].

In addition to reduced birth weight, intrauterine crowding can retard the physiological development of the fetus during gestation [24,25]. Intrauterine growth-restricted (IUGR) piglets are not only physically disproportionate at birth with a 'dolphin-like' head shape [26] but are also compromised metabolically by immature intestinal development [27,28] and an increased disturbance in inflammatory and metabolic profiles [29]. As such, IUGR pigs have a significantly lower capacity for early survival [26,30], and early management should be prioritized to non-IUGR, low-birth-weight pigs [31]. Management strategies for improving survival of low-birth-weight piglets include the provision of an energy source [32], exposure to warmth [33], and assisting with colostrum intake [34]. Low-birth-weight piglets that survive to market weight have been found to have similar carcass quality, meat palatability and prime cut weights as pigs born of normal weight [35]. Prioritizing management towards their survival in larger litters would therefore be worthwhile from not only an ethical standpoint, but an economic one.

2.2. Intrapartum Hypoxia and Farrowing Difficulties

In addition to in utero development, issues can arise for larger litters around farrowing, which can have significant effects on early piglet survival. Problems during the birthing process can lead to an increased incidence of intrapartum hypoxia [36,37]. When a piglet experiences hypoxia during delivery, the concentration of lactate in the

blood rises as ATP must be created in the absence of oxygen [38]. Plush et al. [39] found with every piglet born alive in a litter, the concentration of lactate in piglet cord blood increased by 0.18 ± 0.1 mmol. Intrapartum hypoxia is so hazardous to piglets as even temporary deprivation of oxygen can cause permanent damage to the brain and central nervous system [36]. Mota-Rojas et al. [40] found approximately 14% of live-born piglets in commercial production have reduced viability as a result of experiencing temporary hypoxia during delivery. Low viability piglets are less likely to consume colostrum post-partum and have a greater risk of being overlain by the sow [41]. In addition, piglets that experienced near death hypoxia had abnormal respiratory efforts and cardiac rates [42], which negatively affect early viability. Lucia et al. [43] found sows giving birth to more than 12 pigs were twice as likely to have a stillbirth, and eight times more likely to have a dystocia event requiring manual assistance. Issues arise with larger litters as the farrowing process usually takes longer [42,43], which increases the risk of farrowing difficulties [42]. Peltoniemi et al. [44] observed sows with a farrowing duration over 300 min were twice as likely to have a fetal death during or immediately after birth. Therefore, strategies for reducing farrowing duration in prolific sows should be examined. Along with increased litter size, farrowing can be prolonged in sows of higher parity [45] as well as for those sows experiencing abnormally high levels of stress around parturition [46]. Abnormally high stress and pain responses during parturition increase circulating catecholamines in sows [47]. As natural inhibitors of oxytocin, higher concentrations of catecholamines can potentially slow or stop myometrial contractions [48] and prolong piglet birth intervals to dangerous levels. Further, although an increase in cortisol is necessary for triggering parturition [49], excessive levels of cortisol may lead to issues during farrowing. As both prolonged farrowing duration [13] and increases in litter size [50] have been found to increase circulating levels of cortisol in the sow, it would be important to review how stress can be minimized leading up to and during parturition.

2.3. Increased litter competition and insufficient colostrum intake

Once born, a piglet's survival depends on its ability to effectively compete with littermates for a teat to suckle colostrum [34]. The more piglets that there are in a litter, the greater the competition is for teat access, particularly for piglets of lower birth weight and/or viability [22]. Colostrum is the first secretion of the mammary gland,

characterized by its richness in dry matter and immunoglobulins [51]. These mammary secretions are essential for extrauterine survival as they provide piglets with a source of heat, digestible energy, immunoglobulins and immune cells [52,53]. As the epitheliochorial placenta of a sow does not permit transfer of antibodies, piglets are reliant on colostrum for maternal passive immunity transfer and protection from infection [54]. Absorption of IgG and immune cells by piglets is dependent on timing of gut closure or visceral maturation and the leakiness of the piglet intestinal mucosa [55]. As well as being vulnerable to pathogens [54], piglets have no brown adipose tissue and only a small amount of energy to allow the shivering reflex [55,56]. The minimum net energy required by a 1.0 kg piglet for heat production is between 900 and 1000 kJ on the first day [54]. Although glycogen body reserves can provide some energy, it only amounts to approximately 420 kJ/kg BW [55] and a colostrum intake lower than 140–150 g is insufficient to meet energy requirements. Devillers et al. [51] observed piglets consuming less than 200 g of colostrum had a pre-weaning mortality rate of 43.4%, whereas piglets who consumed over 200 g had a mortality rate as low as 7.1%. In addition, it was found that piglets ingesting less than 290 g of colostrum had a 15% reduction in body weight at weaning [51], a result supported by Quesnel et al. [56] in their review on colostrum intake and piglet performance. Larger litters do not only have a greater proportion of piglets born underweight, but also a greater variance in birth weights within the litter. Le Dividich et al. [54] found colostrum intake in piglets was reduced by 26 ± 1.6 g for every 100 g reduction in birth weight. As colostrum production is not determined by litter size [42] and the fixed volume of colostrum provided by the sow must be shared amongst all piglets, there is a lesser chance of low-birth-weight piglets ingesting an adequate amount of colostrum [57] and they are likely to be outcompeted for teat access by larger littermates [31].

2.4. Increased incidence of piglet crushing by the sow

One of the leading causes of early piglet mortality is the crushing or overlay of piglets by the sow [58]. Across pig breeds, litter size is a contributing factor towards higher crushing incidence [59,60] along with increased sow parity [60], sow movement [58], poorer maternal behaviours [60] and reduced piglet vitality [61]. Sows that experience stress and discomfort during the periparturient period are more likely to move around and increase the likelihood of overlay, especially if the sow 'flops straight down' from a

standing position [58]. As the incidence of sows crushing any piglets was greater for prolific sows, Andersen et al. [62] theorized that the crushing could be a potential strategy to reduce maternal investment in larger litters. As for the piglets, those more susceptible to being overlain are usually weaker and with lower viability [61].

3. Potential strategies for improving survival in large litters.

3.1. Managing colostrum consumption

As low-birth-weight piglets have less energy reserves and a lower capacity for thermoregulation [20,21], they are especially dependent on adequate colostrum intake for survival. Moreira et al. [63] observed the chance of low-birth-weight pigs (800–1200 g) surviving to weaning rose over 89% when they received 200 mL of colostrum (50 mL every 6 h). This finding supports Declerck et al. [34] who found that the correlation between low colostrum intake and reduced pre weaning survival had the greatest effect on piglets in the lower-birth-weight bracket. In addition to its role in early survival, colostrum has a notable effect on the growth and maturation of the neonatal gut [64]. Several bioactive components in colostrum are responsible for activating enzymes along the intestinal brush border and triggering crypt cell proliferation [63–65]. Insulin-like growth factor-1 (IGF-1), one of the compounds responsible for gut maturation, is twice as concentrated in sow colostrum as in milk [66], highlighting the importance of colostrum for both early survival and regular development. In turn, closer management around large litters should focus on colostrum ingestion for both low-birth-weight and low viable piglets. Effective strategies include the split suckling technique [67,68] which allows lower-birth-weight pigs the opportunity to suck by temporarily crating larger piglets [68]. When there were more piglets than functioning teats, i.e., teats that provide adequate volumes of colostrum, the pre-weaning mortality rate in the litter was shown to increase from 8% to 14%. In circumstances where piglets must be fostered off a sow, fostering should occur after colostrum ingestion from the maternal sow but before establishment of teat order by littermates [69]. Deen and Bilkei. [70] observed that low-birth-weight piglets had a greater chance of survival in litters when larger piglets were fostered off, and it is recommended that small piglets remain on the maternal sow [68].

3.2. Inducing sows for increased farrowing supervision

One of the best management strategies for piglet survival in larger litters is adequate farrowing supervision [71]. If sows give birth during working hours, producers are able to effectively save piglets at risk by keeping neonates warm [72,73], rescuing overlain piglets from under sows [74], encouraging suckling behaviours [72], and assisting sows with farrowing difficulties [73]. To allow for this extra supervision, sows can be induced to farrow using prostaglandin (PG) F2α or analogues (e.g., cloprostenol) [74]. The optimal time to induce farrowing is herd specific, but induction should not be performed prior to two days before the herd average gestation length [75]. The main reason for this is that the saccular phase of lung development only occurs during the last two weeks of gestation [76] and inducing too early will result in liveborn pigs with compromised lung function. To improve the likelihood of sows farrowing 22–32 h post treatment, two PGF2α injections should be administered approximately 6 h apart [75], which increases the proportion of sows farrowing the next working day from 55% to 84% [74–76] and thus allows for closer supervision of piglets during and after birth.

3.3. Treating sows with uterotonics during farrowing

A uterotonic such as oxytocin is administered to stimulate uterine contractions to shorten farrowing duration [74,76,77]. Although treating sows with oxytocin may reduce farrowing duration [76], it can cause adverse effects for both sow and piglet. When oxytocin is administered before any piglet had been born, farrowing can be prolonged due to the pain of delivery through a potentially incompletely dilated cervix inducing an acute release of adrenaline, potentially inhibiting further uterine contractions [74]. When the cervix has completely dilated, as indicated by the delivery of the first pig, the administration of oxytocin will reduce farrowing duration but can trigger such powerful and long-lasting uterine contractions that it has been linked with greater fetal stress, intrapartum hypoxia and stillbirth [74,76,78–80]. Lucia et al. [42] found the use of oxytocin during farrowing increased the incidence of stillbirth and its use was not recommended until a minimum of six piglets had been born. An alternative and less potent uterotonic to oxytocin, carbetocin reduces farrowing duration [81–84], reduces piglet hypoxia [81,82] and stillbirth rate [83], but was associated with a reduction in piglet circulating protein concentrations [82] and, presumably, colostrum uptake [84]. Rather than providing an alternate uterotonic to oxytocin, other

management strategies that may improve farrowing performance should be considered to minimize early mortality in larger litters.

3.4. Reducing sow stress to improve farrowing performance

Regardless of the farrowing environment, cortisol concentrations always rise prior to parturition [85]. Although this rise is expected, it is important to evaluate how stress around parturition can be controlled to minimize the risk of farrowing issues. Sows housed in farrowing crates in late gestation have reportedly higher concentrations of plasma cortisol than do sows housed in pens [86] and this may impact farrowing performance [87]. Farrowing crates are commonplace housing for farrowing sows [13] due to their lower space requirements and reduced risk of overlays [88,89]. In comparison to alternative systems like pens, farrowing crates limit the sow's movement to sitting and standing positions, which can increase stress as sows cannot exhibit natural pre-farrowing behaviours [88,89]. As a way of improving sow well-being and possibly piglet survival, alternative farrowing environments have been investigated, as summarized in Table 1.

T**able 1.** Alternative Farrowing environments and their influence on sow and/or piglet performance

Farrowing environments	Observations	Reference
Farrowing crates vs single open pens vs group open pens	-Higher incidence of crushing in loose housing (both single and grouped) within first three days after birth compared to crated sows.	Nicolaisen et al. [90]
Open pens vs crates	-Open penned farrowing increased piglet mortality in three different sow herds	Hales et al. [91]
Open pens vs crates	-Sows housed in open pens had fewer pain related behaviours during farrowing and delivered fewer stillborns	Nowland et al. [50]
Open pens vs crates vs crates for 0-4 days postpartum and then moved into open pen.	-Crating sows for the first four days postpartum was sufficient to reduce piglet mortality compared to farrowing in open pens	Moustsen et al. [92]
Crates that allow 360 degree movement vs conventional crates	-Piglet survival improved in 360 crates only when a sow farrowed in them for their previous farrowing	King et al. [93]
Freedom farrowing pens vs crated sows	-Freedom farrowing pens allow opportunity to nest build and greater movement. Freedom pens reduced rate of stillbirth and farrowing duration but increased crushing incidence	Gu et al. [94]
'Schmidt' pens that provide nesting enrichment vs conventional crates	-Open farrowing pen design showed a tendency towards more crushing but crated sows tended to have a higher incidence of low birthweight piglets	Weber et al. [95]

Although sows in open pen systems have an increased tendency to overlay piglets compared to conventional crates [92,93,95], evidence of improved farrowing performance has been observed [50,94,95]. As suggested by Temple Grandin, even if a

new farrowing environment could reduce sow stress without compromising piglet survival, the costs and space needed to implement new technologies into commercial production often requires '…*more work than doing the research*.' [96]. As such, it is important to review alternative ways to reduce the stress associated with farrowing confinement for sows rearing larger litters in order to improve farrowing performance. According to Grandin and Johnson [97], if you cannot give an animal the freedom to act naturally, then you should think about how to satisfy the emotion that motivates the behaviour. If a suitable nesting environment cannot be provided for the sow, there may be another way to satisfy strong nesting desires for confined sows. Baxter et al. found the provision of a comfortable and flexible lying substrate was enough to 'switch off' nesting behaviour in sows, including pawing and manipulating surrounding substrate [98]. Damm et al. [99] suggested the sight of a perceived 'hollow' in front of a sow's crate is enough to satisfy nesting behaviour. For crated sows, a change in the surrounding environment may show improvements to sow behaviour, but there is currently little evidence of improvement in early piglet survival [100–104].

3.5. Provision of dietary supplements:

During late gestation and lactation, the increased metabolic demand on the sow can elevate the concentration of free radicals and, in turn, the levels of oxidative stress [105]. In addition to negative effects on sow well-being [106], elevated levels of oxidative stress around the periparturient period can impair early lactation output and increase risk of stillbirth [105]. As oxidative stress has been found to increase with litter size [107], it may be a particular concern for hyper prolific sows. The use of supplemental oil with antioxidant properties could be a low-cost strategy for reducing oxidative stress. The supplement's effectiveness in reducing oxidative stress is affected by oil type, oil quality (i.e., is it oxidized) and dosage. A summary of antioxidant-based oil supplements and their effectiveness on sow performance is presented in Table 2.

Table 2. Factors influencing the effectiveness of oil supplements

Comparison	Observations	Reference
Oil type (Fish oil vs Soybean oil)	-Fish oil stimulated greater release of anti-inflammatory compounds and improved rate of pre-weaning survival compared to soybean oil	Yang et al. [108]
Oil type (Fish oil vs Olive oil)	-Olive oil was more effective in reducing oxidative stress, increased milk fat content and improved rate of pre-weaning survival compared to fish oil	Shen et al. [109]
Oil type (Echium oil vs Linseed oil vs Fish oil)	-Fish oil stimulated greater release of anti-inflammatory compounds compared to echium and linseed oils	Tanghe et al. [110]
Supplement (Oregano oil) vs no supplement	-Oregano oil effectively reduced oxidative stress on the first day of lactation and increased feed intake of sows three weeks after farrowing	Tan et al. [111]
Oil quality (fresh vs oxidized)	-Fresh corn oil stimulated greater release of anti-inflammatory agents and more effective reduction in oxidative stress compared to oxidized corn oil	Su et al. [112]
Dosage of oil supplement	-Increasing the dose of fish and linseed oil from 0.5% to 2% stimulated a greater release of anti-inflammatory EPA into sow serum.	Tanghe et al. [113]

Evidently, oils that stimulated greater release of anti-inflammatory compounds and reduced oxidative stress show a positive effect on sow performance [109,111] and

improvements to pre-weaning survival [108,109]. Reducing inflammation can alleviate discomfort in the sow and bring down stress associated with farrowing a large litter of piglets in the confinement of a conventional farrowing crate.

3.6. Provision of anti-inflammatory drugs

Providing an anti-inflammatory drug to peripartum sows would have a more rapid onset of effect than with dietary supplements, and so potentially improve sow and/or piglet performance. Sows experiencing significant discomfort during farrowing show lower circulating concentrations of oxytocin [114] due to elevated cortisol triggering the release of opioids. Lower levels of oxytocin may disrupt rhythmic myometrial contractions [49,115] and thus prolong the delivery of piglets. As well as risk to piglet delivery, pain around parturition may increase the level of agitation and activity displayed by the sow [42]. If anti-inflammatory treatments could diminish this activity, then there would be more opportunities for piglets to suck with a reduced risk of overlay. Anti-inflammatory treatment relieves sow pain by blocking certain stages of the inflammatory process [116]. Treatment can be either non-steroidal (NSAIDs) or steroidal (glucocorticoids), which have different effects on the body due to their different solubility and ability to cross cellular phospholipid bilayers [116,117].

3.6.1. Non-steroidal anti-inflammatory drugs (NSAIDs)

NSAIDs are weak organic acids that cannot pass freely through the phospholipid bilayer and that inhibit cyclooxygenase enzymes (COX I and II) from converting arachidonic acid into pro-inflammatory prostaglandins [117]. Homedes et al. [118] found the NSAID ketaprofen improved piglet survival at days two to seven postpartum and that this effect was most pronounced in large litters. Other studies involving both ketaprofen and meloxican found no evidence of improved piglet survival [119–122]. As colostrum and milk production are limiting factors in larger litters, it has been suggested the benefits of ketaprofen were influenced by the sow's improved milk production [118]. NSAIDs have been found to significantly reduce the incidence of constipation in sows [121], which is important for milk yield due to reduced levels of lipopolysaccharide absorption and increased levels of circulating prolactin [121]. Prolactin is responsible for triggering lactogenesis by stimulating the synthesis of lactose, which is required in high amounts for water to transfer into the mammary alveolar lumen [122]. Further, Mainau et al. [123]

found that although piglet survival rates were not affected, treating sows with NSAIDs post-partum improved IgG concentration in the serum of day-old piglets. NSAID may show improvements to lactation output, but it is not certain whether this translates into improved piglet survival to weaning.

3.6.2. Steroidal anti-inflammatory drugs

Glucocorticoids (GCs) are a class of steroid hormones that assist in reducing inflammation in response to biological stress [124]. The in vivo synthesis and secretion of GCs are regulated by the hypothalamic–pituitary–adrenal axis (HPA) but can also be administered exogenously [125]. As lipid-soluble steroids, GCs diffuse across the cell membrane to stimulate a targeted anti-inflammatory response [126,127]. By passing directly through the cell wall, GCs trigger the 'switching off' of genes that code for pro-inflammatory proteins. Inhibiting the production of chemokines, cytokines and the enzymes COX I and II will reduce inflammation and pain in the target tissue [128,129]. As well as delivering a targeted response, GCs relieve inflammation at multiple sites due to their ability to enter any cell type [130], which is why they are a highly effective anti-inflammatory treatment. Duration, dosage, GC type and mode of application all have an influence on the effect on the anti-inflammatory effect [128]. The type of GC is classified by its potency, with plasma half-lives ranging from 80 min (cortisol) to 270 min (dexamethasone) [126]. In addition to the method of treatment, the effect of GCs on a cell is dependent on the rate of absorption as well as the presence of GC receptors. A potent GC like dexamethasone should be the most effective GC for relieving inflammatory pain that arises with farrowing due to its high potency and long half-life. To exert an effect on genes, GCs must bind to specific receptors present in almost all cells in the mammalian body [125,128] Once activated, GC receptors (GCRs) dissociate from chaperone proteins (e.g., HSP90), translocate to the nucleus and bind to GC-responsive elements for gene expression [126]. In addition to their presence in other organs, GCRs are highly expressed within the placenta [125, 128] as they are important signaling hormones during pregnancy [129]. In early gestation, GCs are responsible for detecting available intrauterine space and modifying fetal development. Towards the end of gestation, a natural rise in GCs triggers the maturation of organs to prepare the fetuses for extra uterine life [129,130]. Although GCs can readily cross the placental barrier, the fetus is protected from GC overexposure by placental 11-β-hydroxysteroid

dehydrogenase type 2 (11-β-HSD-2) [131]. This enzyme facilitates the conversion of most of the maternal cortisol into inert 11-keto forms to ensure the fetus has a far lower level of circulating GCs than the mother [124,131]. The level of GC exposure a fetus receives during gestation will have lifelong consequences to its physiological 'programming' [126,132]. According to Seckl et al. [125], programming refers to the associations between perinatal environmental events and later pathophysiology. Regulating fetal GC exposure is important, as high GC concentrations have been shown to restrict skeletal development, increase the likelihood of IUGR, and impair normal programming responses [109,133]. In humans, treating healthy mothers with dexamethasone during late pregnancy has also shown improvements in the viability of pre-term infants and improves chances of fetal survival [133]. The effects GCs have on the developing fetuses are largely dependent on the timing of treatment, stage of fetal development and level of fetal exposure [123,134]. Treating crated sows with glucocorticoids prior to farrowing has potential to not only improve well-being of the sow, but the survival and future growth of piglets. The treatment of sows with glucocorticoids prior to farrowing has the potential to reduce the pain and inflammatory response, thus resulting in improved sow well-being and lactation performance. Due to the ability of glucocorticoids to advance the maturation of visceral organs, glucocorticoids used in conjunction with farrowing induction may also reduce the risk of underdeveloped viscera (e.g., lungs and gastrointestinal system) and improve piglet preweaning growth and survival. However, the appropriate timing and duration of treatment is currently unknown.

4. Conclusion

Based on the literature reviewed, it is evident that larger litters provide significant challenges for sow and litter management. The current management paradigm is essentially an economic one, with profit being the primary motivation. The development of hyper prolific sows fits with the economic paradigm as the modern sow weans more pigs available for market. However, perhaps we need a more ethical paradigm and should not be asking how many pigs are weaned, but rather how many pigs died in order to achieve the numbers weaned. The increased risk of low-birth-weight piglets with greater weight variation within the litter makes it very difficult for the smaller piglets to survive to weaning, and many require significant attention and supervision to survive. On many, if not most, sow farms, the necessary supervision is not available.

Longer farrowing durations, an increased incidence of stillbirth and intrapartum hypoxia, inadequate colostrum intake and overlain piglets are issues that need to be addressed as the industry evolves to accommodate larger litters. Additionally, understanding that hyperprolific sows have a heightened metabolic demand, greater susceptibility to oxidative stress, usually longer farrowing duration with greater levels of discomfort, and an increased tendency to overlay piglets will all be important for current and future production. Continued research into strategies that will reduce sow stress and allow them opportunities to display natural nesting behaviour and/or movement without the risk of overlay, show signs of improvement in both farrowing and lactation performance. As production science evolves, it is important that the needs of both the41 Animals 2020, 10, 309 9 of 15 sow and piglets are considered. Evidence-based management protocols that show improvements to piglet survival, and that may or may not involve pharmaceutical intervention, should be implemented

Author Contributions: All authors were involved in the conceptualization and the writing and review of all draft. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding. Acknowledgments: Sophia Ward gratefully acknowledges the University of Adelaide and Australian Pork Limited for their provision of a scholarship.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Luković, Z.; Škorput, D. Factors influencing litter size in pigs. *CAB Rev.* **2015**, *10*, 1-9.
2. Kemp, B.; Da Silva, C.L.; Soede, N.M. Recent advances in pig reproduction: Focus on impact of genetic selection for female fertility. *Zuchthygiene* **2018**, *53*, 28-36.
3. Zak, L.J.; Gaustad, A.H.; Bolarin, A.; Broekhuijse, M.L.; Walling, G.A.; Knol, E.F. Genetic control of complex traits, with a focus on reproduction in pigs. *Mol. Reprod. Dev.* **2017**, *84*, 1004-1011.
4. Gerritsen, R.; Soede, N.; Langendijk, P.; Taverne, M.; Kemp, B. Early embryo survival and development in sows with lactational ovulation. *Reprod. Domest. Anim.* **2008**, *43*, 59-65.
5. Yuan, T.L.; Zhu, Y.H.; Shi, M.; Li, T.T.; Li, N.; Wu, G.Y.; Wang, J.J. Within-litter variation in birth weight: Impact of nutritional status in the sow. J. Zhejiang Univ. Sci. B. 2015, 16, 417–435.
6. Yuanfeng, L.; He, J.; Ao, X.; Wang, Y. Effect of dietary nutrition on litter size born alive of sows. *Feed Ind.* **2017**, *20*, 6-8.
7. Quiniou, N.; Dagorn, J.; Gaudré, D. Variation of piglets' birth weight and consequences on subsequent performance. *Livest. Prod. Sci.* **2002**, *78*, 63-70.

78. Schmitt, O.; Baxter, E.M.; Lawlor, P.G.; Boyle, L.A.; O'Driscoll, K.A. single dose of fat-based energy supplement to light birth weight pigs shortly after birth does not increase their survival and growth. *Animal* **2019**, *9*, 227.
9. Pardo, C.; Bérard, J.; Kreuzer, M.; Bee, G. Intrauterine crowding impairs formation and growth of secondary myofibers in pigs. *Animal* **2013**, *7*, 430-438.
10. Madsen, J.; Bee, G. Compensatory growth feeding strategy does not overcome negative effects on growth and carcass composition of low birth weight pigs. *Animal.* **2015**, *9*, 427-436.
11. Langendijk, P.; Chen, T.; Athorn, R.; Bouwman, E.G. Embryonic survival at day 9, 21 and 35 of pregnancy in intact and unilaterally oviduct ligated multiparous sows. *Animal* **2016**, *10*, 1336-1341.
12. Greenwood, P.L.; Bell, A.W.; Vercoe, P.E.; Viljoen, G.J. Quantification of prenatal effects on productivity in pigs. In *Managing the Prenatal Environment to Enhance Livestock Productivity*; Springer: Dordrecht, The Netherlands, 2010; pp. 37-69.
13. Roelofs, S.; Godding, L.; de Haan, J.R.; van der Staay, F.J.; Nordquist, R.E. Effects of parity and litter size on cortisol measures in commercially housed sows and their offspring. *Physiol.Behav.* **2019**, *201*, 83-90.
14. Fijn, L.; Antonides, A.; Aalderink, D.; Nordquist, R.E.; van der Staay, F.J. Does litter size affect emotionality, spatial learning and memory in piglets? *Appl. Anim. Behav. Sci.* **2016**, *178*, 23-31.
15. Van der Waaij, E.; Hazeleger, W.; Soede, N.; Laurenssen, B.; Kemp, B. Effect of excessive, hormonally induced intrauterine crowding in the gilt on fetal development on day 40 of pregnancy. *J. Anim. Sci.* **2010**, *88*, 2611-2619.
16. Wolf, J.; Žáková, E.; Groeneveld, E. Within-litter variation of birth weight in hyperprolific Czech Large White sows and its relation to litter size traits, stillborn piglets and losses until weaning. *Livest. Sci.* **2008**, *115*, 195-205.
17. Lund, M.; Puonti, M.; Rydhmer, L.; Jensen, J. Relationship between litter size and perinatal and pre-weaning survival in pigs. *Anim. Sci.* **2002**, *74*, 217-222.
18. Baxter, E.; Rutherford, K.; D'eath, R.; Arnott, G.; Turner, S.; Sandøe, P.; Moustsen, V.; Thorup, F.; Edwards, S.; Lawrence, A.B. The welfare implications of large litter size in the domestic pig II: management factors. *Anim. Welf.* **2013**, *22*, 219-238.
19. Tuchscherer, M.; Puppe, B.; Tuchscherer, A.; Tiemann, U. Early identification of neonates at risk: traits of newborn piglets with respect to survival. *Theriogenology.* **2000**, *54*, 371-388.
20. Milligan, B.N.; Fraser, D.; Kramer, D.L. Within-litter birth weight variation in the domestic pig and its relation to pre-weaning survival, weight gain, and variation in weaning weights. *Livest. Prod. Sci.* **2002**, *76*, 181-191.
21. Zeng, Z.K.; Urriola, P.E.; Dunkelberger, J.R.; Eggert, J.M.; Vogelzang, R.; Shurson, G.C.; Johnston, L.J. Implications of early-life indicators for survival rate, subsequent growth performance, and carcass characteristics of commercial pigs. *J. Anim. Sci.* **2019**, *97*, 3313-3325.
22. Orihuela, A.; Solano, J.J. Managing "teat order" in suckling pigs (Sus scrofa domestica). *Appl. Anim. Behav. Sci.* **1995**, *46*, 125-130.
23. Škorput, D.; Dujmović, Z.; Karolyi, D.; Luković, Z. Variability of birth weight and growth of piglets in highly prolific sows. *J. Cent. Eur. Agric.* **2018**, *19*, 823-828.
24. Amdi, C.; Krogh, U.; Flummer, C.; Oksbjerg, N.; Hansen, C.; Theil, P.K. Intrauterine growth restricted piglets defined by their head shape ingest insufficient amounts of colostrum. *J. Anim. Sci.* **2013**, *91*, 5605-5613.
25. Wang, X.; Wu, W.; Lin, G.; Li, D.; Wu, G.; Wang, J. Temporal proteomic analysis reveals continuous impairment of intestinal development in neonatal piglets with intrauterine growth restriction. *J. Proteome Res.* **2010**, *9*, 924-935.

26. Hales, J.; Moustsen, V.; Nielsen, M.; Hansen, C.F. Individual physical characteristics of neonatal piglets affect preweaning survival of piglets born in a noncrated system. *J. Anim. Sci.* **2013**, *91*, 4991-5003.
27. Wang, J.; Chen, L.; Li, D.; Yin, Y.; Wang, X.; Li, P.; Dangott, L.J.; Hu, W.; Wu, G. Intrauterine growth restriction affects the proteomes of the small intestine, liver, and skeletal muscle in newborn pigs. *J. Nutr.* **2008**, *138*, 60-66.
28. Hansen, C.; Hales, J.; Amdi, C.; Moustsen, V.A. Intrauterine growth-restricted piglets defined by their head shape have impaired survival and growth during the suckling period. *Anim. Prod. Sci.* **2018**, *59*, 1056-1062.
29. Huang, S.; Li, N.; Liu, C.; Li, T.; Wang, W.; Jiang, L.; Li, Z.; Han, D.; Tao, S.; Wang, J. Characteristics of the gut microbiota colonization, inflammatory profile, and plasma metabolome in intrauterine growth restricted piglets during the first 12 hours after birth. *J. Microbiol.* **2019**, *57*, 748-758.
30. Engelsmann, M.N.; Hansen, C.F.; Nielsen, M.N.; Kristensen, A.R.; Amdi, C. Glucose injections at birth, warmth and placing at a nurse sow improve the growth of IUGR piglets. *Animals.* **2019**, *9*, 519.
31. Rutherford, K.; Baxter, E.; D'eath, R.; Turner, S.; Arnott, G.; Roehe, R.; Ask, B.; Sandøe, P.; Moustsen, V.; Thorup, F. Edwards, S.A. The welfare implications of large litter size in the domestic pig I: biological factors. *Anim. Welf.* **2013**, *22*, 199-218.
32. Bikker, P.; Kranendonk, G.; Gerritsen, R.; Russell, L.; Campbell, J.; Crenshaw, J.; Rodríguez, C.; Ródenas, J.; Polo, J. Absorption of orally supplied immunoglobulins in neonatal piglets. *Livest. Sci.* **2010**, *134*, 139-142.
33. Caldara, F.R.; Dos Santos, L.S.; Machado, S.T.; Moi, M.; de Alencar Nääs, I.; Foppa, L.; Garcia, R.G.; Dos Santos, R.D. Piglets' surface temperature change at different weights at birth. *Asian-Australas. J. Anim. Sci.* **2014**, *27*, 431.
34. Declerck, I.; Dewulf, J.; Sarrazin, S.; Maes, D. Long-term effects of colostrum intake in piglet mortality and performance. *J. Anim. Sci.* **2016**, *94*, 1633-1643.
35. Beaulieu, A.; Aalhus, J.; Williams, N.; Patience, J.F. Impact of piglet birth weight, birth order, and litter size on subsequent growth performance, carcass quality, muscle composition, and eating quality of pork. *J. Anim. Sci.* **2010**, *88*, 2767-2778.
36. Mota-Rojas, D.; Rosales, A.; Trujillo, M.; Orozco, H.; Ramirez, R.; Alonso-Spilsbury, M. The effects of vetrabutin chlorhydrate and oxytocin on stillbirth rate and asphyxia in swine. *Theriogenology.* **2005**, *64*, 1889-1897.
37. Langendijk, P.; Plush, K. Parturition and its relationship with stillbirths and asphyxiated piglets. *Animals.* **2019**, *9*, 885.
38. Kraut, J.A.; Madias, N.E. Lactic acidosis. *N. Engl. J. Med.* **2014**, *371*, 2309-2319.
39. Plush, K.; Weaver, A.; Staveley, L.; Van Wettere, W. Maternal magnesium sulfate supplementation in a pre-farrow diet improves factors important for piglet viability. *Animals.* **2018**, *8*, 185.
40. Alonso-Spilsbury, M.; Mota-Rojas, D.; Villanueva-García, D.; Martínez-Burnes, J.; Orozco, H.; Ramírez-Necoechea, R.; Mayagoitia, A.L.; Trujillo, M.E. Perinatal asphyxia pathophysiology in pig and human: a review. *Anim. Reprod. Sci.* **2005**, *90*, 1-30.
41. Mota-Rojas, D.; Martínez-Burnes, J.; Trujillo-Ortega, M.E.; Alonso-Spilsbury, M.L.; Ramírez-Necoechea, R.; López, A. Effect of oxytocin treatment in sows on umbilical cord morphology, meconium staining, and neonatal mortality of piglets. *Am. J. Vet. Res.* **2002**, *63*, 1571-1574.
42. Oliviero, C.; Junnikkala, S.; Peltoniemi, O. The challenge of large litters on the immune system of the sow and the piglets. *Reprod. Domest. Anim.* **2019**, *54*, 12-21.
43. Lucia Jr, T.; Corrêa, M.N.; Deschamps, J.C.; Bianchi, I.; Donin, M.A.; Machado, A.C.; Meincke, W.; Matheus, J.E. Risk factors for stillbirths in two swine farms in the south of Brazil. *Prev. Vet. Med.* **2002**, *53*, 285-292.
44. Peltoniemi, O.; Oliviero, C. Housing, management and environment during farrowing and early lactation. *The Gestating and Lactating Sow*, 77-84. **2015**, 231.

45. Lavery, A.; Lawlor, P.; Magowan, E.; Miller, H.; O'driscoll, K.; Berry, D.P. An association analysis of sow parity, live-weight and back-fat depth as indicators of sow productivity. *Animal* **2019**, *13*, 622-630.
46. Merlot, E.; Pastorelli, H.; Prunier, A.; Père, M.C.; Louveau, I.; Lefaucheur, L.; Quesnel, H. Sow environment during gestation: Part I. Influence on maternal physiology and lacteal secretions in relation with neonatal survival. *Animal* **2019**, *13*, 1432–1439.
47. Von Borell, E.; Dobson, H.; Prunier, A. Stress, behaviour and reproductive performance in female cattle and pigs. *Horm. Behav.* **2007**, *52*, 130–138.
48. Yun, J.; Valros, A. Benefits of prepartum nest-building behaviour on parturition and lactation in sows—A review. *Asian Australas. J. Anim. Sci.* **2015**, *28*, 1519.
49. Algers, B.; Uvnäs-Moberg, K. Maternal behavior in pigs. *Horm. Behav.* **2007,** 52, 78–85.
50. Nowland, T.L.; van Wettere, W.H.E.J.; Plush, K.J. Allowing sows to farrow unconfined has positive implications for sow and piglet welfare. *Appl. Anim. Behav. Sci.* **2019**, *221*, 104872.
51. Devillers, N.; Le Dividich, J.; Prunier, A. Influence of colostrum intake on piglet survival and immunity. *Animal* **2011,** *5*, 1605–1612.
52. Martin, M.; Tesouro, M.; González-Ramón, N.; Piñeiro, A.; Lampreave, F. Major plasma proteins in pig serum during postnatal development. *Reprod. Fertil. Dev.* **2005**, *17*, 439–445.
53. Vasdal, G.; Andersen, I.L. A note on teat accessibility and sow parity—Consequences for newborn piglets. *Livest. Sci.* **2012**, *146*, 91–94.
54. Le Dividich, J. The issue of colostrum in piglet survival: Energy and immunity. In *Nutritional Biotechnology in the Feed and Food Industries, Proceedings of the Alltech's 22nd Annual Symposium, Lexington, KY, USA, 23–26 April 2006*; Lyons, T.P., Jacques, K.A., Hower, J.M., Eds.; Alltech: Stamford, UK, 2006.
55. Rooke, J.A.; Bland, I.M. The acquisition of passive immunity in the new-born piglet. *Livest. Prod. Sci*. **2002**, *78*, 13–23.
56. Quesnel, H.; Farmer, C.; Devillers, N. Colostrum intake: Influence on piglet performance and factors of variation. *Livest. Sci.* **2012**, 146, 105–114.
57. Herpin, P.; Damon, M.; Le Dividich, J. Development of thermoregulation and neonatal survival in pigs. *Liv. Prod. Sci.* **2002**, *78*, 25–45.
58. Andersen, I.L.; Berg, S.; Bøe, K.E. Crushing of piglets by the mother sow (Sus scrofa)—Purely accidental or a poor mother? *Appl. Anim. Behav. Sci.* **2005**, *93*, 229–243.
59. Ocepek, M.; Newberry, R.C.; Andersen, I.L. Trade-offs between litter size and offspring fitness in domestic pigs subjected to different genetic selection pressures. *Appl. Anim. Behav. Sci.* **2017**, *193*, 7–14.
60. Weary, D.M.; Phillips, P.A.; Pajor, E.A.; Fraser, D.; Thompson, B.K. Crushing of piglets by sows: Effects of litter features, pen features and sow behaviour. *Appl. Anim. Behav. Sci.* **1998**, *61*, 103–111.
61. Muns, R.; Nuntapaitoon, M.; Tummaruk, P. Non-infectious causes of pre-weaning mortality in piglets. *Livest. Sci.* **2016**, *184*, 46–57.
62. Andersen, I.L.; Nævdal, E.; Bøe, K.E. Maternal investment, sibling competition, and offspring survival with increasing litter size and parity in pigs (Sus scrofa). *Behav. Ecol. Sociobiol.* **2011**, *65*, 1159–1167.
63. Moreira, L.P.; Menegat, M.B.; Barros, G.P.; Bernardi, M.L.; Wentz, I.; Bortolozzo, F.P. Effects of colostrum, and protein and energy supplementation on survival and performance of low-birth-weight piglets. *Livest. Sci.* **2017**, *202*, 188–193.
64. Jensen, A.R.; Elnif, J.; Burrin, D.G.; Sangild, P.T. Development of intestinal immunoglobulin absorption and enzyme activities in neonatal pigs is diet dependent. *J. Nutr.* 2001, 131, 3259–3265.
65. Xu, R.; Sangild, P.T.; Zhang, Y.; Zhang, S. Bioactive compounds in porcine colostrum and milk and their effects on intestinal development in neonatal pigs. *Biol. Grow. Anim.* **2002**, *1*, 169–192.

66. Schmitt, O.; Baxter, E.M.; Boyle, L.A.; O'Driscoll, K. Nurse sow strategies in the domestic pig: II. Consequences for piglet growth, suckling behaviour and sow nursing behaviour. *Animal* **2019,** *13*, 590–599.
67. Farmer, C.; Devillers, N.; Rooke, J.A.; Dividich, J.L. Colostrum production in swine: From the mammary glands to the piglets. *Pig News Inf.* **2006**, 27, 1–16.
68. Alexopoulos, J.G.; Lines, D.S.; Hallett, S.; Plush, K.J. A review of success factors for piglet fostering in lactation. *Animals* **2018**, *8*, 38.
69. Heim, G.; Mellagi, A.P.G.; Bierhals, T.; de Souza, L.P.; De Fries, H.C.C.; Piuco, P.; Bortolozzo, F.P. Effects of cross-fostering within 24 h after birth on pre-weaning behaviour, growth performance and survival rate of biological and adopted piglets. *Livest. Sci.* **2012**, *150*, 121–127.
70. Deen, M.G.H.; Bilkei, G. Cross fostering of low-birthweight piglets. *Livest. Prod. Sci.* **2004**, *90*, 279–284.
71. Hoshino, Y.; Koketsu, Y. Assessment of post-farrowing performance in sows having a high number of pigs born dead on commercial farms. *Livest. Sci.* **2009**, *125*, 244–248.
72. Vande Pol, K.D.; Cooper, N.; Tolosa, A.; Ellis, M.; Shull, C.M.; Brown, K.; Alencar, S. PSV-4 Effect of drying and/or warming piglets at birth on rectal temperature over the first 24 h after birth. *J. Anim. Sci*. **2019**, *97*, 157–158.
73. Lawlor, P.G.; Lynch, P.B. Management interventions to help keep piglets alive in large litters. *Ir. Vet. J.* **2005**, *58*, 640–645.
74. Vila, R.M.; Tummaruk, P. Management strategies in farrowing house to improve piglet pre-weaning survival and growth. *Thai J. Vet. Med.* **2016**, *46*, 347–354.
75. Decaluwe, R.; Janssens, G.; Declerck, I.; de Kruif, A.; Maes, D. Induction of parturition in the sow. *Vlaams Diergen. Tijds*. **2012**, *81*, 158–165.
76. Kirkwood, R.N.; Aherne, F.X. Increasing the predictability of cloprostenol-induced farrowing in sows. *J. Swine Health Prod.* ***1998,*** *6,* 57–59
77. Kirkwood, R.N. Induction of parturition in sows. *Thai J. Vet. Med.* **2015**, *45*, 487–491
78. Cassar, G.; Kirkwood, R.N.; Friendship, R.; Poljak, Z. Sow and litter performance following farrowing induction with prostaglandin: Effect of adjunct treatments with dexamethasone or oxytocin. *J. Swine Health Prod.* **2005**, 13, 81–85.
79. De Rensis, F.; Saleri, R.; Tummaruk, P.; Techakumphu, M.; Kirkwood, R. Prostaglandin F2α and control of reproduction in female swine: A review. *Theriogenology* **2012**, *77*, 1–11.
80. Mota-Rojas, D.; Martínez-Burnes, J.; Trujillo, M.E.; López, A.; Rosales, A.M.; Ramírez, R.; Alonso-Spilsbury, M. Uterine and fetal asphyxia monitoring in parturient sows treated with oxytocin. *Anim. Reprod. Sci.* **2005**, *86*, 131–141
81. Linneen, S.K.; Benz, J.M.; DeRouchey, J.M.; Goodband, R.D.; Tokach, M.D.; Dritz, S.S. A review of oxytocin use for sows and gilts. *Kans. Aes. Res. Publ.* **2005**, 10, 1–3.
82. Ward, S.A.; Kirkwood, R.N.; Plush, K.L. Effects of oxytocin and carbetocin on farrowing performance. *Anim. Reprod. Sci.* **2019**, *205*, 88–93.
83. Boonraungrod, N.; Sutthiya, N.; Kumwan, P.; Tossakui, P.; Nuntapaitoon, M.; Muns, R.; Tummaruk, P. Control of parturition in swine using PGF2α in combination with carbetocin. *Livest. Sci.* **2018**, *214*, 1–8.
84. Udomchanya, J.; Suwannutsiri, A.; Sripantabut, K.; Pruchayakul, P.; Juthamanee, P.; Nuntapaitoon, M.; Tummaruk, P. Association between the incidence of stillbirths and expulsion interval, piglet birth weight, litter size and carbetocin administration in hyper-prolific sows. *Livest. Sci.* **2019**, *227*, 128–134.
85. Jiarpinitnun, P.; Loyawatananan, S.; Sangratkanjanasin, P.; Kompong, K.; Nuntapaitoon, M.; Muns, R.; Tummaruk, P. Administration of carbetocin after the first piglet was born reduced farrowing duration but compromised colostrum intake in newborn piglets. *Theriogenology* **2019**, *128*, 23–30.

86. Yun, J.; Swan, K.-M.; Vienola, K.; Farmer, C.; Oliviero, C.; Peltoniemi, O.; Valros, A. Nest-building in sows: Effects of farrowing housing on hormonal modulation of maternal characteristics. *Appl. Anim. Behav. Sci.* **2013**, *148*, 77–84.
87. Wischner, D.; Kemper, N.; Krieter, J. Nest-building behaviour in sows and consequences for pig husbandry. *Livest. Sci.* **2009**, *124*, 1–8.
88. Blackshaw, J.K.; Blackshaw, A.W.; Thomas, F.J.; Newman, F.W. Comparison of behaviour patterns of sows and litters in a farrowing crate and a farrowing pen. *App. Anim. Behav. Sci*. **1994**, *39*, 281–295.
89. Vrbanac, I.; Balenovi´c, T.; Yammine, R.; Valpoti´c, I.; Krsnik, B. Preweaning losses of piglets on a state farm in Bosnia and Herzegovina. *Prev. Vet. Med.* **1995**, *24*, 23–30.
90. Nicolaisen, T.; Lühken, E.; Volkmann, N.; Rohn, K.; Kemper, N.; Fels, M. The Effect of Sows' and Piglets' Behaviour on Piglet Crushing Patterns in Two Different Farrowing Pen Systems. *Animal* **2019**, *9*, 538.
91. Hales, J.; Moustsen, V.A.; Nielsen, M.B.F.; Hansen, C.F. Higher preweaning mortality in free farrowing pens compared with farrowing crates in three commercial pig farms. *Animal* **2014**, 8, 113–120.
92. Moustsen, V.A.; Hales, J.; Lahrmann, H.P.; Weber, P.M.; Hansen, C.F. Confinement of lactating sows in crates for 4 days after farrowing reduces piglet mortality. *Animal* **2013**, *7*, 648–654.
93. King, R.L.; Baxter, E.M.; Matheson, S.M.; Edwards, S.A. Consistency is key: Interactions of current and previous farrowing system on litter size and piglet mortality. *Animal* **2019**, *13*, 180–188.
94. Gu, Z.; Gao, Y.; Lin, B.; Zhong, Z.; Liu, Z.; Wang, C.; Li, B. Impacts of a freedom farrowing pen design on sow behaviours and performance. *Prev. Vet. Med.* **2011**, *102*, 296–303.
95. Weber, R. Alternative housing systems for farrowing and lactating sows. *Pub. Eur. Assoc. Anim. Prod.* **2000,** *102*, 109–116.
96. Grandin, T. Transferring results of behavioral research to industry to improve animal welfare on the farm, ranch and the slaughter plant. *Appl. Anim. Behav. Sci.* **2003**, *81*, 215–228.
97. Grandin, T.; Johnson, C. *Animals Make Us Human: Creating the Best Life for Animals*, 1st ed.; Houghton Mifflin Harcourt Publishing Company: New York, NY, USA, 2009; p. 2.
98. Baxter, E.; Rutherford, K.; d'Eath, R.; Arnott, G.; Turner, S.; Sandøe, P.; Lawrence, A. The welfare implications of large litter size in the domestic pig II: Management factors. *Anim. Welf.* **2013**, *22*, 219–238.
99. Damm, B.; Bildsøe, M.; Gilbert, C.; Ladewig, J.; Vestergaard, K. The effects of confinement on periparturient behaviour and circulating prolactin, prostaglandin F2α and oxytocin in gilts with access to a variety of nest materials. *Appl. Anim. Behav. Sci.* **2002**, *76*, 135–156.
100. Cronin, G.M.; Simpson, G.J.; Hemsworth, P.H. The effects of the gestation and farrowing environments on sow and piglet behaviour and piglet survival and growth in early lactation. *Appl. Anim. Behav. Sci.* **1996**, *46*, 175–192.
101. Jarvis, S.; Lawrence, A.B.; McLean, K.A.; Deans, L.A.; Chirnside, J.; Calvert, S.K. The effect of environment on behavioral activity, ACTH, (β-endorphin and cortisol in pre-farrowing gilts. *Anim. Sci.* **1997**, *65*, 465–472.
102. Baxter, E.M.; Jarvis, S.; Sherwood, L.; Robson, S.K.; Ormandy, E.; Farish, M.; Edwards, S.A. Indicators of piglet survival in an outdoor farrowing system. *Livest. Sci.* **2009**, *124*, 266–276.
103. Wechsler, B.; Weber, R. Loose farrowing systems: Challenges and solutions. *Anim. Welf.* **2017**, *16*, 295–307.
104. Kecman, J.; Waehner, M. Management of large litters in piglet production. *Tierarztl. Prax. Ausg. G Grosstiere* **2016**, *44*, 318–325.

105. Berchieri-Ronchi, C.B.; Kim, S.W.; Zhao, Y.; Correa, C.R.; Yeum, K.J.; Ferreira, A.L.A. Oxidative stress status of highly prolific sows during gestation and lactation. *Animal* **2011**, *5*, 1774–1779.
106. Fan, Z.; Xiao, Y.; Chen, Y.; Wu, X.; Zhang, G.; Wang, Q.; Xie, C. Effects of catechins on litter size, reproductive performance and antioxidative status in gestating sows. *Anim. Nutr.* **2015**, *1*, 271–275.
107. Liu, K.; Wu, W.; Li, B.; Wei, W.; Liu, Z.; Liu, H.L. Changes in Oxidative Stress Status in Sows from Days 100 of Gestation to Post-Partum Estrus. *Pak. Vet. J.* **2018**, *38*, 165–168.
108. Yang, Y.; Hu, C.J.; Zhao, X.; Xiao, K.; Deng, M.; Zhang, L.; Tan, C. Dietary energy sources during late gestation and lactation of sows: Effects on performance, glucolipid metabolism, oxidative status of sows, and their offspring. *J. Anim. Sci.* **2019**, *97*, 4608–4618.
109. Shen, Y.; Wan, H.; Zhu, J.; Fang, Z.; Che, L.; Xu, S.; Wu, D. Fish oil and olive oil supplementation in late pregnancy and lactation differentially affect oxidative stress and inflammation in sows and piglets. *Lipids* **2015**, *50*, 647–658.
110. Tanghe, S.; Millet, S.; De Smet, S. Echium oil and linseed oil as alternatives for fish oil in the maternal diet: Blood fatty acid profiles and oxidative status of sows and piglets. *J. Anim. Sci.* **2013**, *91*, 3253–3264.
111. Tan, C.; Wei, H.; Sun, H.; Ao, J.; Long, G.; Jiang, S.; Peng, J. Effects of dietary supplementation of oregano essential oil to sows on oxidative stress status, lactation feed intake of sows, and piglet performance. *BioMed. Res. Int.* **2015**, 525218.
112. Su, G.; Zhao, J.; Luo, G.; Xuan, Y.; Fang, Z.; Lin, Y.; Che, L. Effects of oil quality and antioxidant supplementation on sow performance, milk composition and oxidative status in serum and placenta. *Lipids Health Dis*. **2017**, *16*, 107.
113. Tanghe, S.; Missotten, J.; Raes, K.; De Smet, S. The effect of different concentrations of linseed oil or fish oil in the maternal diet on the fatty acid composition and oxidative status of sows and piglets. *J. Anim. Physiol. Anim. Nutr.* **2015**, *99*, 938–949.
114. Rezaei, R.; Wu, Z.; Hou, Y.; Bazer, F.W.; Wu, G. Amino acids and mammary gland development: Nutritional implications for milk production and neonatal growth. *J. Anim. Sci. Biotech*. **2016**, *7*, 20.
115. Fviero, C.; Heinonen, M.; Valros, A.; Hälli, O.; Peltoniemi, O. Effect of the environment on the physiology of the sow during late pregnancy, farrowing and early lactation. *Anim. Reprod. Sci*. **2008**, *105*, 365–377.
116. Mainau, E.; Ruiz-de-la-Torre, J.; Dalmau, A.; Salleras, J.; Manteca, X. Effects of meloxicam (Metacam®) on post-farrowing sow behaviour and piglet performance. *Animals* **2012**, *6*, 494–501.
117. Becker, D.E. Basic and clinical pharmacology of glucocorticosteroids. *Anesth. Prog*. **2013**, *60*, 25–32
118. Homedes, J.; Salichs, M.; Sabaté, D.; Sust, M.; Fabre, R. Effect of ketoprofen on pre-weaning piglet mortality on commercial farms. *Vet. J.* **2014**, *201*, 435–437.
119. Tenbergen, R.; Friendship, R.; Cassar, G.; Amezcua, M.; Haley, D. Investigation of the use of meloxicam post farrowing for improving sow performance and reducing pain. *J. Swine Health Prod.* **2014**, *22*, 10–15.
120. Claeyé, E.; Beek, J.; Meyns, T.; Maes, D. Effect of ketoprofen treatment in the prevention of postpartum dysgalactia syndrome in sows. *Vlaams Diergen. Tijds.* **2015**, *84*, 127–132.
121. Viitasaari, E.; Hänninen, L.; Heinonen, M.; Raekallio, M.; Orro, T.; Peltoniemi, O.; Valros, A. Effects of post-partum administration of ketoprofen on sow health and piglet growth. *Vet. J.* **2013,** *198*, 153–157.
122. Lawrence, A.B.; Petherick, J.C.; McLean, K.A.; Deans, L.A.; Chirnside, J.; Gaughan, A.; Terlouw, E.M.C. The effect of environment on behaviour, plasma cortisol and prolactin in parturient sows. *Appl. Anim. Behav. Sci*. **1994**, *39*, 313–330.

123. Mainau, E.; Temple, D.; Manteca, X. Experimental study on the effect of oral meloxicam administration in sows on pre-weaning mortality and growth and immunoglobulin G transfer to piglets. *Prev. Vet. Med.* **2016**, *126*, 48–53.
124. Busada, J.T.; Cidlowski, J.A. Mechanisms of glucocorticoid action during development. In *Current Topics in Developmental Biology*; Academic Press: Cambridge, MA, USA, 2017; Volume 125, pp. 147–170.
125. Seckl, J.R.; Nyirenda, M.J.; Walker, B.R.; Chapman, K.E. Glucocorticoids and fetal programming. *Biochem. Soc. Trans.* **1999**, *27*, 74–78.
126. Romundstad, L.; Stubhaug, A. Glucocorticoids for acute and persistent postoperative neuropathic pain: What is the evidence? *Anesthesiology* **2007**, *107*, 371–373.
127. Barnes, P.J.; Adcock, I.M. Glucocorticoid resistance in inflammatory diseases. *Lancet* **2009**, *373*, 1905–1917.
128. De Bosscher, K.; Berghe, W.V.; Haegeman, G. Mechanisms of anti-inflammatory action and of immunosuppression by glucocorticoids: Negative interference of activated glucocorticoid receptor with transcription factors. *J. Neurol*. **2000**, *109*, 16–22.
129. Schäcke, H.; Döcke, W.D.; Asadullah, K. Mechanisms involved in the side effects of glucocorticoids. *Pharmacol. Ther.* **2002**, *96*, 23–43.
130. Newton, R. Molecular mechanisms of glucocorticoid action: What is important? *Thorax* **2000**, *55*, 603–613.
131. Sliwa, E.; Dobrowolski, P.; Piersiak, T. Bone development of suckling piglets after prenatal, neonatal or ´ perinatal treatment with dexamethasone. *J. Anim. Physiol. Anim. Nutr.* **2010**, *94*, 293–306.
132. Fowden, A.L.; Li, J.; Forhead, A.J. Glucocorticoids and the preparation for life after birth: Are there long-term consequences of the life insurance? *Proc. Nutr. Soc.* **1998**, *57*, 113–122.
133. Moisiadis, V.G.; Matthews, S.G. Glucocorticoids and fetal programming part 1: Outcomes. *Nat. Rev. Endocrinol.* **2014**, *10*, 391.
134. Chen, M.; Wang, T.; Liao, Z.X.; Pan, X.L.; Feng, Y.H.; Wang, H. Nicotine-induced prenatal overexposure to maternal glucocorticoid and intrauterine growth retardation in rat. Exp. *Toxicol. Pathol.* **2007**, *59*, 245–251

Chapter 2: Administering dexamethasone to prepartum sows: effects on sow and piglet performance.

Statement of Authorship

Title of Paper	Administering dexamethasone to prepartum sows: effects on sow and piglet performance.
Publication Status	☒ Published ☐ Accepted for publication ☐ Submitted for publication ☐ Unpublished and Unsubmitted work written in manuscript style
Publication Details	Ward, S. A., Kirkwood, R. N., & Plush, K. J. (2020). Administering dexamethasone to prepartum sows: Effects on sow and piglet performance. Livestock Science, 239, 104171. DOI: https://doi.org/10.1016/j.livsci.2020.104171

Principal Author

Name of Principal Author (Candidate)	Sophia Ward		
Contribution to the Paper	Designed experimental methodology, carried out experimental procedures, analyses statistics, wrote first manuscript.		
Overall percentage (%)	80 %		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	22/02/2022

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Roy Kirkwood		
Contribution to the Paper	Conception of the theme, assisted with design of experimental methodology and drafting of the manuscript (10%)		
Signature		Date	22/02/2022
Name of Co-Author	Kate Plush		
Contribution to the Paper	Assisted with statistical analysis, drafting and evaluation of manuscript (10%)		
Signature		Date	22/02/2022

Livestock Science 239 (2020) 104171

Contents lists available at ScienceDirect

Livestock Science

journal homepage: www.elsevier.com/locate/livsci

Article

Administering dexamethasone to prepartum sows: effects on sow and piglet performance.

Sophia A. Ward[1]*, Roy N. Kirkwood[1], Kate J. Plush[2]

[1] School of Animal and Veterinary Sciences, University of Adelaide, SA, Australia
[2] Sunpork Group, Shea-Oak Log, SA 5371, Australia

Received 9 April 2020, Revised 16 July 2020, Accepted 16 July 2020, Available online 17 July 2020

Abstract

There is a considerable gap in available literature regarding the use of prepartum steroidal anti-inflammatory drugs (SAIDs) despite their ability to provide a targeted response to pain. The aim of this investigation was to determine whether prepartum administration of dexamethasone to sows would influence farrowing and/or lactation processes, with the prediction that it would affect both sow and piglet performance. Sows were induced to farrow on day 113 of gestation (2 days before their 115 day due date) and were treated with dexamethasone concurrent with induction (DexInd; n = 20), 24 h after induction (Dex24; n = 20), at induction and 24 h later (Dextwice; n = 19) or no steroid treatment (Control; n = 20). Treating with dexamethasone at induction reduced birth and weaning weights (P = 0.001), plasma total protein concentrations (P = 0.001), and colostrum intake of piglets of multiparous sows (P = 0.006). Treating primiparous sows with dexamethasone 24 h after induction did not adversely affect piglet weights or plasma total protein and improved average daily gain in piglets (P = 0.001). Although no differences were observed for farrowing duration or piglet survival (P > 0.05), further investigation is required using higher parity sows to qualify whether glucocorticoids can improve farrowing performance.

Keywords: sow; farrowing; colostrum; piglets; preweaning mortality.

1. Introduction

Parturition can be a painful process [1] and the provision of analgesia may allow sows to cope with farrowing and/or lactation processes more effectively. Administering non-steroidal anti-inflammatory drugs (NSAIDs) to sows has reported effects on sow performance [2-4] but results are yet to show an improvement to piglet growth and/or survival. Unlike NSAIDs, glucocorticoids (GCs) pass directly through the phospholipid bilayer of cells and stimulate an anti-inflammatory response directly within the tissue [5,6]. After passing through the cell membrane, GC's trigger the 'switching off' of genes that code for pro-inflammatory proteins in the nucleus, including chemokines and leukotrienes [5-7]. As well as delivering a more targeted response, GCs relieve inflammation at multiple sites due to their ability to enter any cell type [6] which is why they are an effective anti-inflammatory treatment [7]. Pain-induced stress during parturition can increase the concentrations of catecholamines in sow plasma [9], which have the potential to inhibit myometrial contractions and increase complications with the farrowing process [1]. If sows were treated with a long-lasting GC like dexamethasone prior to farrowing, the relief from pain during delivery may reduce the incidence of complications and the incidence of piglet hypoxia or stillbirth [10].

Administering dexamethasone to sows prior to farrowing has the potential to not only improve farrowing performance, but also colostrum quality and quantity. Colostrum is the first secretion of the mammary gland and, in addition to the provision of passive immunity, is essential for providing piglets with heat and digestible energy to cope with extrauterine life [11]. As dexamethasone can reduce inflammation in multiple sites, it may reduce the level of proinflammatory cytokine TNF-α, which is known to disrupt lactose synthesis and inactivate milk secreting cells [12].

In addition to sow performance, administering dexamethasone to sows pre-partum may have an effect on piglet visceral maturation. Natural GCs are normally regulated from passing through placental barriers in high amounts due to chaperone proteins (placental 11-βhydroxysteroid dehydrogenase type 2) but synthetic GCs are not picked up as readily [13]. Regulating fetal GC exposure is important as high GC concentrations have been shown to restrict skeletal development, increase the likelihood of intrauterine growth restriction and impair normal programming responses [14-17]. The effects GCs have on the developing fetuses are largely dependent on the timing of treatment, stage of fetal development and level of fetal exposure [16,18]. It was hypothesized that by

administering dexamethasone to sows at different times pre-partum, sow and piglet performance will be affected in different ways.

2. Materials and Methods

2.1. Animals and management

This study was performed in four batch farrowings with an equal spread of parity and treatment from August 2018 to February 2019 at The University of Adelaide research piggery with the approval of The University of Adelaide ethics committee (approval number S2018–038). A total of 79 Large White x Landrace sows (parities one to five) were moved into individual farrowing crates one week before expected due dates. Sows were fed twice daily with a commercial diet formulated to meet all nutrient requirements and had free access to fresh water. At 113 days of gestation (2 days before their 115-day due date) sows were induced to farrow with 125 μg prostaglandin analogue, cloprostenol (Juramate®, Jurox Pty, Ltd, Rutherford, NSW, Australia), administered at 0700 h and again at 1300 h so that farrowing would occur on day 114 of gestation. Only clinically healthy sows delivering their first piglet between 0730 and 1730 h on day 114 were included in the study.

2.2. Treatment

At the time of first cloprostenol injection, sows were assigned to treatment by parity so as to minimize mean parity differences and received an intramuscular injection [20 mg] of dexamethasone (Dexapent®, Troy Laboratories, Glendenning, NSW, Australia) either with induction (DexInd; n = 20), 24 h after induction (Dex24; n = 20), with induction and 24 h later (Dextwice; n = 19), or no steroid treatment following induction (Control; n = 20). A timeline of induction and treatments is presented in Figure 1.

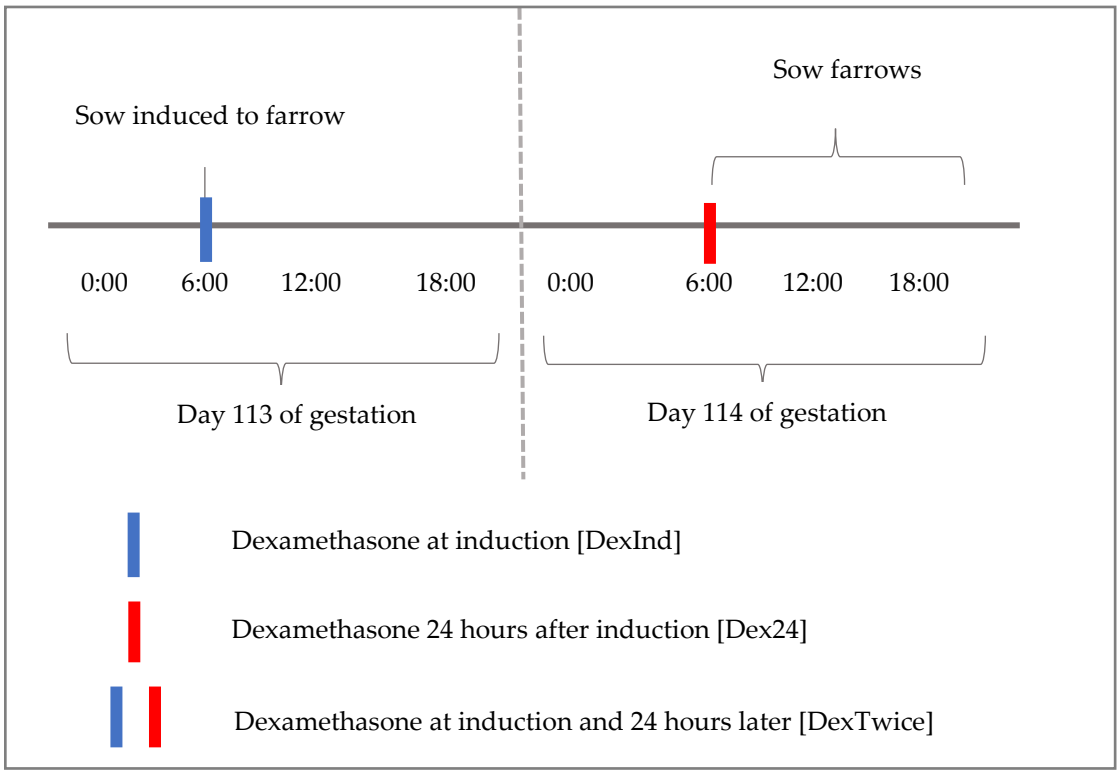


Figure 1. Timeframe outlining the times in which sows were induced to farrow, and treatments were administered according to the expected 115-day gestation period. On day 113, two days before the intended due date, sows were given an inducing agent so the following day they would farrow (one day before intended due date). Treatments were administered either with the inducing agent (7am on day 113: DexInd), 24 h after the inducing agent (7am on day 114: Dex24), both times (DexTwice) or no steroid treatment (Control).

2.3. Data collection

Farrowing duration, incidences of dystocia, stillbirths and overlays were recorded as markers of farrowing performance. Obstetric assistance was provided for sows if piglet delivery intervals exceeded 40 min, and each intervention recorded as a dystocia event. At birth of the first and ninth piglet, colostrum protein (%) was measured using a Brix refractometer [19]. As strong correlations have been observed between Brix refractometry values and total concentration of IgG protein in both serum and colostrum [19,20] total protein was determined using a digital Brix refractometer. Upon delivery, piglets were recorded as liveborn or stillborn, and live piglets weighed and placed back in their original position behind the sow. At 24 h post farrowing, piglets were weighed again and their colostrum intake between birth and 24 h calculated using the following formula [21]:

$$CI = -217.4 + 0.217*t + 1861019*BW/t + BWb*(54.8 - 1861019/t) * (0.9985 - 3.7*10^{-4}\,tfs + 6.1 * 10^{-7} * t^2fs$$

Where *CI* = colostrum intake (g), *BW* = piglet body weight at 24 h, BW_b = piglet body weight at birth, *t* = time elapsed from birth to first suckling (min).

Devillers et al. [21] proposed that the interval of elapsed time from birth to first suckling can be estimated as between 15 and 30 min without major error. In this study, the average interval of time was chosen to be 20 min. Total colostrum yield of the sow was estimated as the sum of colostrum intakes of all piglets in the litter.

At 24 h post farrowing, piglets 1, 2, 3, 8, 9 and 10 were blood sampled (3 mL) via vena cava puncture. Using an on-farm centrifuge (3000 rpm x 10 mins) plasma was extracted from blood samples and assessed for total protein content (%) using a handheld PAL-IOS™ refractometer (Atago PAL-11S, Starr Instruments, Dandenong South, Vic, Australia). Based on the 98.8% correlation determined between refractometer values and the total protein percentage of samples [22], refractometry data are referred to as plasma protein (%). At day 18, piglet preweaning survival was recorded and piglets were weighed again.

2.4. Statistical analysis

Data were analyzed using IBM SPSS v20. Data were analyzed for normal distribution prior to assessment with the exception of binary measurements. Incidences of stillbirth and dystocia, and piglet preweaning mortality were analyzed with binary logistic regression with the random term replicate and fixed effects parity group (P1 or P>1), treatment, and first order interactions. Sow data were assessed using a linear mixed model with the random term replicate and fixed effects parity group (P1 or P>1), treatment, and first order interactions. For piglet data, repeated measure analyses were used with sow identification as the subject and birth order number as the repeated measure. Replicate was fit as a random term, with treatment, gender, parity group, litter size group (14, large), birth order group (1–4, first; 5–9, middle; >9, last) and birth weight group (1.6 kg, heavy) as fixed effects, with two-way interactions fitted. Litter size was fit as a covariate for both sow and piglet data. These results were analyzed and presented as means ± standard error of the mean.

3. Results

The average parity of sows was 1.88 ± 0.20, and the mean litter size was 11.8 ± 0.33, with neither trait different between treatments. Administering dexamethasone to prepartum sows had no effect on the duration of farrowing ($P = 0.8$), incidence of dystocia ($P = 1.00$), stillbirth ($P = 1.00$), overlay ($P = 0.12$), preweaning mortality ($P = 0.167$) or colostrum yield ($P = 0.61$) (Table 1). Additionally, dexamethasone did not affect the concentration of protein in colostrum either at farrowing onset or at the birth of the ninth piglet (Table 1). Administering dexamethasone 24 h after induction [Dextwice, Dex24] did, however, result in a greater concentration of protein in colostrum compared to sows treated with dexamethasone only at induction ($P = 0.17$; Table 1).

Table 1: Dex administered either with the inducing agent (7am on day 113; DexInd); 24 h after the inducing agent (7am on day 144; Dex24) both times (DexTwice) or no steroid treatment (Control) on mean (± SE) sow farrowing performance and piglet survival to weaning

	Control	**Dex24**	**Dextwice**	**DexInd**	***P* value**
Farrowing duration (mins)	132 ± 15	149 ± 15	148 ± 13	136 ± 15	0.798
Total born	11.2 ± 8	11.4± 0.8	11.7 ± 0.7	11.8 ± 0.8	0.934
Born alive	10.9 ± 0.7	11.3 ± 0.7	11.3 ± 0.7	11.5 ± 0.7	0.943
Dystocia events (%)	0.4 ± 0.5	2.0 ± 1.1	1.9 ± 1.0	2.0 ± 0.5	1.000
Stillbirths (%)	2.5 ± 1.1	2.0 ± 1.0	2.9 ± 1.1	3.1 ± 1.1	1.000
Incidence of overlay (%)	5.3 ± 2.3	2.1 ± 1.0	6.3 ± 2.1	8.3 ± 3.0	0.122
Preweaning mortality (%)	11.1 ± 2.2	7.1 ± 2.3	12.4 ± 2.3	14.2 ± 2.2	0.167
Sow colostrum volume (kg)	3.92 ± 0.27	4.16 ± 0.27	3.68 ± 0.25	3.77 ± 0.28	0.604
Protein (%) of colostrum at farrowing onset	26.6^{ab} ± 0.6	27.8^{a} ± 0.5	28.1^{a} ± 0.6	25.6^{b} ± 0.6	0.017
Protein (%) of colostrum at birth of ninth piglet	25.2^{ab} ± 0.6	26.4^{a} ± 0.5	26.5^{a} ± 0.6	24.5^{b} ± 0.6	0.043
Brix (%) of piglet plasma at 24 hours post-partum	6.69^{a} ± 0.13	6.79^{a} ± 0.15	6.15^{b} ± 0.19	6.21^{b} ± 0.18	0.026

Further, piglet plasma total protein at 24 h postpartum was lower when sows were treated with dexamethasone at induction [Dextwice, DexInd]. For multiparous sows, the DexInd treatment also resulted in piglets with lower colostrum intake ($P = 0.006$; Figure 2).

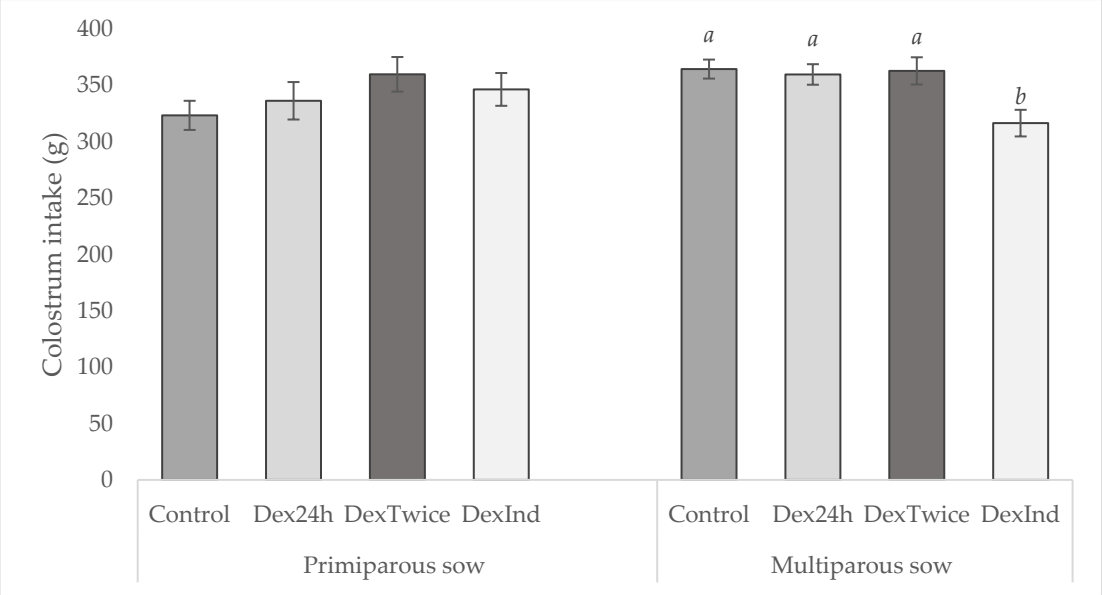


Figure 2. Effects of dexamethasone administered a day before farrowing (day 114 of gestation at 7am) [DexInd], the day of farrowing (day 115 of gestation at 7am) [Dex24], day before (day 114 of gestation at 7am) and on the day of farrowing (day 115 of gestation at 7am) [Dextwice], or no steroid treatment [Control] on mean (±SE) colostrum intake of piglets. Within parity groups, means having different superscripts differ ($P < 0.05$).

Administering dexamethasone to sows with induction and 24 h after had a negative effect on piglet birthweight for both primiparous sows and multiparous sows ($P = 0.001$ for both; Figure 3).

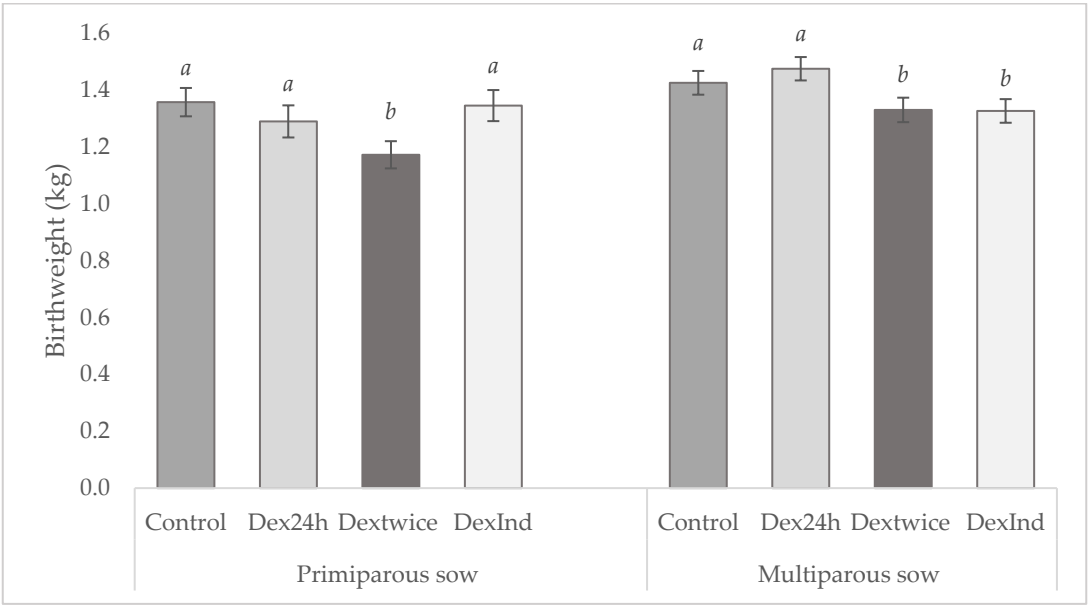


Figure 3. Effects of dexamethasone administered a day before farrowing (day 114 of gestation at 7am) [DexInd], the day of farrowing (day 115 of gestation at 7am) [Dex24], day before (day 114 of gestation at 7am) and on the day of farrowing (day 115 of gestation at 7am) [Dextwice], or no steroid treatment [Control] on mean (±SE) piglet birthweights. Within parity groups, means having different superscripts differ ($P < 0.05$).

Piglets from multiparous sows that received dexamethasone at induction [DexInd, Dextwice] also had lower weights at day 18 ($P = 0.002$; Figure 4).

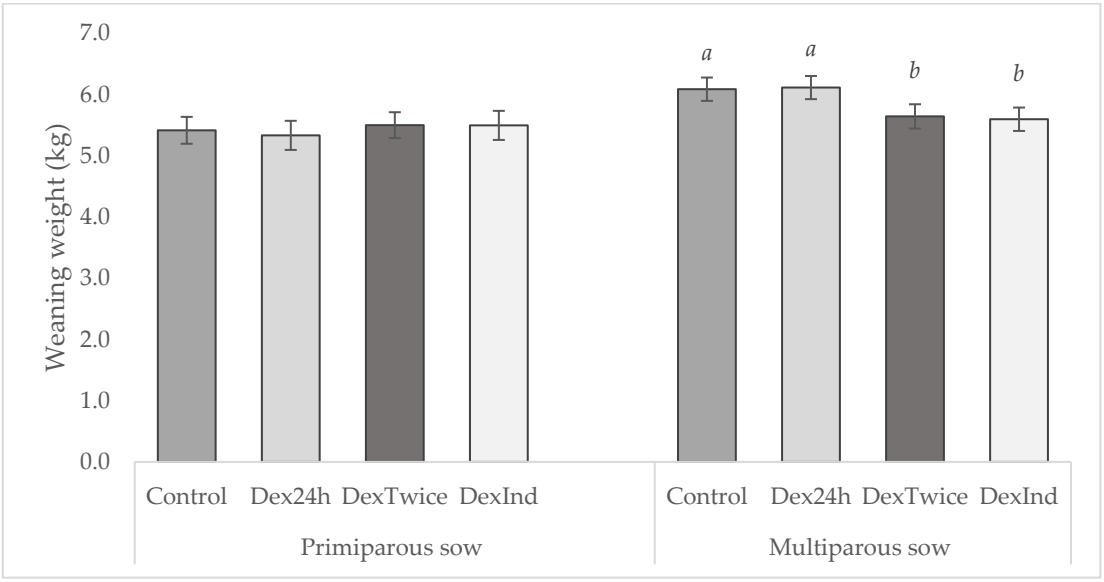


Figure 4. Effects of dexamethasone administered a day before farrowing (day 114 of gestation at 7am) [DexInd], the day of farrowing (day 115 of gestation at 7am) [Dex24], day before (day 114 of gestation at 7am) and on the day of farrowing (day 115 of gestation at 7am) [Dextwice], or no steroid treatment [Control] on mean (±SE) piglet weights at day 18. Within parity groups, means having different superscripts differ ($P < 0.05$).

Primiparous sows that received dexamethasone 24 h after induction [Dex24] had piglets with increased daily gain and multiparous sows that received dexamethasone with induction [DexInd; DexTwice] had piglets with reduced daily gain ($P = 0.026$; Figure 5).

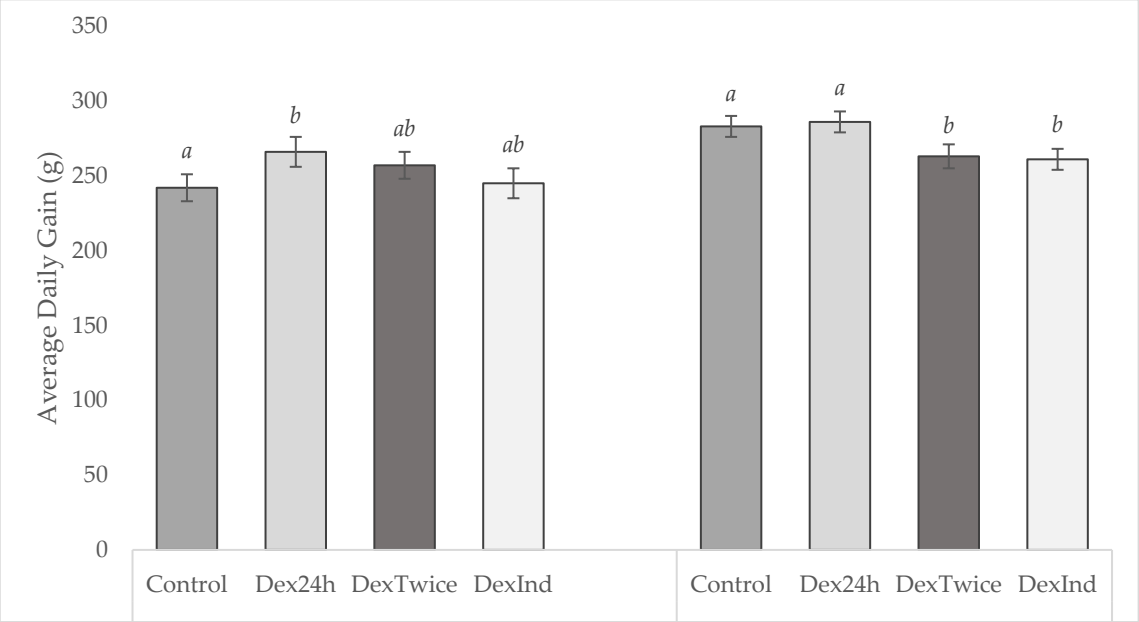


Figure 5. Effects of dexamethasone admnistered a day before farrowing (day 114 of gestation at 7am) [DexInd], the day of farrowing (day 115 of gestation at 7am) [Dex24], day before (day 114 of gestation at 7am) and on the day of farrowing (day 115 of gestation at 7am) [Dextwice], or no steroid treatment [Control] on mean (±SE) average daily gain to day 18. Within parity groups, means having different superscripts differ ($P < 0.05$).

4. Discussion

Dexamethasone both mediates inflammatory processes in sows and potentially affects fetal visceral maturation. Therefore, it is important to examine its effectiveness when administered to sows at different times prior to farrowing. The results of this study suggest dexamethasone can affect aspects of sow and piglet performance, but timing in relation to the day of farrowing is a critical factor in the outcome. It was identified that dexamethasone administered to sows concurrent with induction (the day before farrowing) resulted in negative impacts on both birth and weaning weights. However, when given on the day of farrowing, no adverse outcomes were observed on either sow or piglet, indeed, there was some evidence of improved piglet survival.

In this study, no treatment differences were observed for farrowing duration, stillbirths or need for manual assistance due to dystocia. As a targeted and potent relief from

inflammatory pain, dexamethasone was predicted to reduce the incidence of farrowing complications that would arise from stress, especially in sows with particularly long and painful farrowings [23]. Across treatments, farrowing duration and the incidence of dystocia and stillbirth were low, presumably due to most sows being of a lower parity. Sows of higher parity (P5+) are more likely to experience longer piglet birth intervals and difficulty during the farrowing process due to weakened uterine tone [9]. Additionally, there may have not been issues during the farrowing process as the average litter size was only between 10 and 11 piglets. Sows giving birth to more than 12 piglets were more likely to experience prolonged farrowing duration [8] and were eight times more likely to experience a dystocia event requiring manual assistance [10]. Farrowing difficulties may have been more apparent and potentially responsive to treatment if the average parity and/or litter size was higher.

As glucocorticoids are important mediators of lactogenesis [24], it was predicted dexamethasone would actively contribute to lactation processes and affect colostrum quality. However, dexamethasone did not significantly affect the concentration of protein in colostrum compared to control sows. Macrina et al. [25] found administering dexamethasone to heifers at lactation onset increased mammary cell differentiation but did not improve the concentration of protein in colostrum. In another study with ewes [26], the provision of dexamethasone seven days before parturition prematurely activated lactation and reduced colostral IgG concentrations. Considering sows from the DexInd treatment group had similar concentrations of protein in colostrum compared to controls, it is unlikely DexInd was impairing colostrum quality. By this time in late gestation, natural surges of maternal GCs may have already triggered lactogenesis and there was nothing for exogenous GC treatment to activate. Between treatments, administering dexamethasone the day before farrowing resulted in lower protein concentration in colostrum than when dexamethasone was administered the day of farrowing. Although glucocorticoids can trigger increases in colostrum production [26] stimulating greater lactogenic activity can also have an inverse effect on IgG concentrations because the proteins are more diluted in colostral secretions [27]. Considering estimated colostrum yields were not affected by treatment in this study, it is reasonable to assume another reason for the change in protein concentration.

The tendency towards fewer overlays in Dex24 litters and overall preweaning mortalities may indicate reduced stress and associated sow movements. However, in

the absence of sow behavioral monitoring, this suggestion remains speculative. Machado-Neto et al. [28] found reduced cortisol concentrations in sow colostrum was correlated with an increase in colostral IgG concentrations. Since dexamethasone and cortisol have the same biological activities, an increase in dexamethasone may cause the release of ACTH to be reduced and, in turn, the release of cortisol to be reduced [29]. Future investigations should measure cortisol content in colostrum to see whether provision of dexamethasone actively reduces this stress hormone.

The administration of dexamethasone one day before farrowing was found to reduce piglet birthweight. These results are consistent with other sow studies [30-32] as well as in other species including humans [14] rats [33] and sheep [34]. Whirledge et al. [35] proposed that the reason dexamethasone restricts fetal growth is the result of a 'trade off' in energy reserves. Being a glucocorticoid, dexamethasone can prioritize partitioning of energy towards organ maturation for early survival over skeletal growth. This may have been the case in our study as piglets from both DexInd and DexTwice, although smaller at birth, had the same survival rates as the control piglets. Treating primiparous sows with dexamethasone at induction only reduced birthweights when it was also given again the following day [DexTwice]. Repeating a course of steroid treatment during gestation has shown evidence of growth disturbances in animal and human studies [36] but the data should be interpreted with caution given the short period between dexamethasone injection and piglet delivery. Differences between the effects of dexamethasone at induction and parity groups may be the result of primiparous control sows having already small piglets. Sows from DexInd treatment had similar birth and weaning weights between parity groups, but they were only significantly lower when compared to the piglets from multiparous control sows.

Administering dexamethasone to sows at induction may also impair piglet capacity to ingest and/or absorb macromolecules. Sows treated with dexamethasone at induction and 24 h after [DexTwice] had piglets with reduced protein absorption over sows treated on the day of farrowing [Dex24h], even though colostrum quality was not impaired between these treatment groups. Dexamethasone has been found to alter intestinal permeability in piglets, although treatment was administered to 7-day old piglets [30]. As the intestine of a newborn piglet is considerably different to that of a 7-day old [37] further investigation would be required to understand whether dexamethasone can affect intestinal absorption of piglets prior to gut closure.

For multiparous litters, the intake of colostrum was impaired in piglets from DexInd sows. Consequently, the lower protein absorption of piglets may be a result of ingesting smaller volumes of colostrum. Piglets from the DexInd sows were physically smaller on average than other treatment groups, and a lower birthweight is usually accompanied with a reduced vitality and ability to suck [38]. As there were no differences in piglet pre-weaning mortality across the treatments, the protein absorption of piglets in the DexInd group were evidently still sufficient for survival. When dexamethasone was administered to sows on the day of farrowing it had no negative effects on piglet birthweight, colostrum intake or protein absorption. In primiparous sows, it also improved the daily gain of piglets to weaning. De Rensis et al. [31] observed an improvement to piglet growth rates when sows were treated with dexamethasone, but only for the first three days postpartum. Improved growth rates were also seen when 7-day old piglets were injected with dexamethasone directly rather than exposed via maternal treatment [39]. The reason for this increase in growth may be due to GCs involvement with growth hormone (GH). In studies where elevated GC levels resulted in reduced skeletal growth, the concentration of GH in these animals remained normal [40]. In young rabbits, dexamethasone treatment caused a tissue-specific stimulation of GH receptor mRNA levels [40]. The effects of dexamethasone in this circumstance were dose responsive as lower doses of dexamethasone resulted in greater increases in GH receptor mRNA levels [40].

5. Conclusion

Treating sows with dexamethasone prepartum may improve sow and piglet performance but only when administered 24 h after an induction protocol. Further investigation into treating sows with dexamethasone at this time is recommended as it shows promise of improving daily growth of piglets to weaning. Administering corticosteroids to sows before the day of parturition is not recommended as we have evidence of reduced birthweight, colostrum intake, and protein absorption of piglets. Therefore, taken together this implies that dexamethasone treatment of clinically normal sows should only be considered in association with farrowing induction. However, the impact of prepartum dexamethasone administration to sows considered to be at risk of puerperal disease, indicated by a farm history of farrowing problems, is worthy of investigation.

Declaration of Competing Interest

The authors declare no conflict of interest.

Acknowledgements

We gratefully acknowledge the University of Adelaide and Australian Pork Limited for their financial support.

References

1. Mainau, E.; Manteca, X. Pain and discomfort caused by parturition in cows and sows. Appl. Anim. Behav. Sci. 2011, 135, 241-251.
2. Viitasaari, E.; Hänninen, L.; Heinonen, M.; Raekallio, M.; Orro, T.; Peltoniemi, O.; Valros, A. Effects of post-partum administration of ketoprofen on sow health and piglet growth. Vet. 2013, 198, 153-157.
3. Tenbergen, R.; Friendship, R.; Cassar, G.; Amezcua, M.; Haley, D. Production. Investigation of the use of meloxicam post farrowing for improving sow performance and reducing pain. J. Swine Health Prod. 2014, 22, 10-15.
4. Claeyé, E.; Beek, J.; Meyns, T.; Maes, D. Effect of ketoprofen treatment in the prevention of postpartum dysgalactia syndrome in sows. Vlaams Diergeneeskd Tijdschr. 2015, 84, 127-132.
5. Romundstad, L.; Stubhaug, A.Glucocorticoids for acute and persistent postoperative neuropathic pain: what is the evidence? Anesthesiology. 2007, 107, 371-373.
6. Barnes, P.J.; Adcock, I.M. Glucocorticoid resistance in inflammatory diseases. Lancet. 2009, 373, 1905-1917.
7. De Bosscher, K.; Berghe, W.V.; Haegeman, G. Mechanisms of anti-inflammatory action and of immunosuppression by glucocorticoids: negative interference of activated glucocorticoid receptor with transcription factors. J. Neuroimmunol. 2000, 109, 16-22.
8. Baxter, E.; Rutherford, K.; D'eath, R.; Arnott, G.; Turner, S.; Sandøe, P.; Moustsen, V.; Thorup, F.; Edwards, S.; Lawrence, A.B. The welfare implications of large litter size in the domestic pig II: management factors. Anim. Welf. 2013, 22, 219-238.
9. Oliviero, C.; Junnikkala, S.; Peltoniemi, O. The challenge of large litters on the immune system of the sow and the piglets. Reprod. Domest. Anim. 2019, 54, 12-21.
10. Van Dijk, A.; Van Rens, B.; Van der Lende, T.; Taverne, M.A.M. Factors affecting duration of the expulsive stage of parturition and piglet birth intervals in sows with uncomplicated, spontaneous farrowings. Theriogenology, 2005, 64, 1573-1590.
11. Dividich, J.L. The issue of colostrum in piglet survival: energy and immunity. In Proceedings of the Nutritional biotechnology in the feed and food industries: Proceedings of Alltech's 22nd Annual Symposium, Lexington, Kentucky, USA, 23-26 April 2006, 2006; pp. 89-102.
12. Kobayashi, K.; Kuki, C.; Oyama, S.; Kumura, H. Pro-inflammatory cytokine TNF-α is a key inhibitory factor for lactose synthesis pathway in lactating mammary epithelial cells. Exp. Cell. Res. 2016, 340, 295-304.
13. Seckl, J.; Nyirenda, M.; Walker, B.; Chapman, K.E. Glucocorticoids and fetal programming. Biochem. Soc. Trans. 1999, 27, 74-78.
14. Holemans, K.; Aerts, L.; Van Assche, F.A. Fetal growth restriction and consequences for the offspring in animal models. J. Soc. Gyneol. Investig. 2003, 10, 392-399.
15. Chen, M.; Wang, T.; Liao, Z.X.; Pan, X.L.; Feng, Y.H.; Wang, H. Pathology, T. Nicotine-induced prenatal overexposure to maternal glucocorticoid and intrauterine growth retardation in rat. Exp. Toxicol. Pathol. 2007, 59, 245-251.

16. Gokulakrishnan, G.; Estrada, I.J.; Sosa Jr, H.A.; Fiorotto, M.L. In Utero Glucocorticoid (GLC) Exposure Reduces Fetal Skeletal Muscle Growth in Rats. Am. J. Physiol. Regul. 2010, 24, 740-743.
17. Tan, Y.; Liu, J.; Deng, Y.; Cao, H.; Xu, D.; Cu, F.; Lei, Y.; Magdalou, J.; Wu, M.; Chen, L. Caffeine-induced fetal rat over-exposure to maternal glucocorticoid and histone methylation of liver IGF-1 might cause skeletal growth retardation. Toxicol. Lett. 2012, 214, 279-287.
18. Drake, A.J.; Tang, J.I.; Nyirenda, M.J. Mechanisms underlying the role of glucocorticoids in the early life programming of adult disease. Clin. Sci. 2007, 113, 219-232.
19. Balzani, A.; Cordell, H.; Edwards, S.A. Evaluation of an on-farm method to assess colostrum IgG content in sows. Animal 2016, 10, 643-648.
20. Hasan, S.; Junnikkala, S.; Valros, A.; Peltoniemi, O.; Oliviero, C. Validation of Brix refractometer to estimate colostrum immunoglobulin G content and composition in the sow. Animal 2016, 10, 1728-1733.
21. Devillers, N.; van Milgen, J.; Prunier, A.; Le Dividich, J. Estimation of colostrum intake in the neonatal pig. Anim. Sci. 2004, 78, 305-313.
22. Manjarín, R.; Montano, Y.A.; Kirkwood, R.N.; Bennet, D.C.; Petrovski, K.R. Effect of piglet separation from dam at birth on colostrum uptake. Can. J. Vet. Res. 2018, 82, 239-242.
23. Lucia Jr, T.; Corrêa, M.N.; Deschamps, J.C.; Bianchi, I.; Donin, M.A.; Machado, A.C.; Meincke, W.; Matheus, J.E. Risk factors for stillbirths in two swine farms in the south of Brazil. Prev. Vet. Med. 2002, 53, 285-292.
24. Chida, D.; Miyoshi, K.; Sato, T.; Yoda, T.; Kikusui, T.; Iwakura, Y. The role of glucocorticoids in pregnancy, parturition, lactation, and nurturing in melanocortin receptor 2-deficient mice. Endocrinology 2011, 152, 1652-1660.
25. Macrina, A. L.; Kauf, A. C. W.; Pape-Zambito, D. A.; Kensinger, R. S. Induced lactation in heifers: Effects of dexamethasone and age at induction on milk yield and composition. *J. Dairy. Sci.* **2014**, *97(3)*, 1446-1453.
26. Dawe, S.; Husband, A.; Langford, C.M. Effects of induction of parturition in ewes with dexamethasone or oestrogen on concentrations of immunoglobulins in colostrum, and absorption of immunoglobulins by lambs. Aust. J. Biol. Sci. 1982, 35, 223-230.
27. Guy, M.; McFadden, T.; Cockrell, D.; Besser, T.E. Regulation of colostrum formation in beef and dairy cows. J. Dairy Sci. 1994, 77, 3002-3007.
28. Machado-Neto, R.; Graves, C.N.; Curtis, E. Immunoglobulins in piglets from sows heat-stressed prepartum. J. Anim. Sci. 1987, 65, 445-455.
29. Jovanovic, T.; Phifer, J.E.; Sicking, K.; Weiss, T.; Norrholm, S.D.; Bradley, B.; Ressler, K.J. Cortisol suppression by dexamethasone reduces exaggerated fear responses in posttraumatic stress disorder. Psychoneuroendocrinology 2011, 36, 1540-1552.
30. Weiler, H.A.; Wang, Z.; Atkinson, S.A. Whole body lean mass is altered by dexamethasone treatment through reductions in protein and energy utilization in piglets. Neonatology 1997, 71, 53-59.
31. De Rensis, F.; Sottocorona, M.; Kirkwood, R.N. Effect of prostaglandin and dexamethasone injection on farrowing and piglet neonatal growth. Effect of prostaglandin and dexamethasone injection on farrowing and piglet neonatal growth Vet. 2002, 151, 330-331.
32. Tomaszewska, E.; Dobrowolski, P.; Puzio, I. Postnatal administration of 2-oxoglutaric acid improves the intestinal barrier affected by the prenatal action of dexamethasone in pigs. Nutrition 2012, 28, 190-196.
33. Tan, Y.; Liu, J.; Deng, Y.; Cao, H.; Xu, D.; Cu, F.; Wang, H.; Caffeine-induced fetal rat over-exposure to maternal glucocorticoid and histone methylation of liver IGF-1 might cause skeletal growth retardation. *Toxicol. Lett.***2012**, 214(3), 279-287.

34. Jellyman, J.K.; Martin-Gronert, M.S.; Cripps, R.L.; Giussani, D.A.; Ozanne, S.E.; Shen, Q.W.; Du, M.; Fowden, A.L.; Forhead, A.J. Effects of cortisol and dexamethasone on insulin signalling pathways in skeletal muscle of the ovine fetus during late gestation. *PLoS One,* **2012**, *7*, e52363.
35. Whirledge, S.; Cidlowski, J.A. A role for glucocorticoids in stress-impaired reproduction: beyond the hypothalamus and pituitary. *Endocrinology* **2013**, *154*, 4450-4468.
36. Newnham, J.; Moss, T. Antenatal glucocorticoids and growth: single versus multiple doses in animal and human studies. In Proceedings of the Seminars in Neonatology, 2001; pp. 285-292.
37. Rooke, J.; Bland, I.M. The acquisition of passive immunity in the new-born piglet. *Livest. Prod. Sci.* **2002**, *78*, 13-23.
38. Alexopoulos, J.G.; Lines, D.S.; Hallett, S.; Plush, K.J. A review of success factors for piglet fostering in lactation. *Animals* **2018**, *8*, 38.
39. Carroll, J.A. Dexamethasone treatment at birth enhances neonatal growth in swine. *Domest. Anim. Endocrinol.* **2001**, *21*, 97-109.
40. Heinrichs, C.; Yanovski, J.; Roth, A.H.; Yu, Y.; Domene, H.; Yano, K.; Cutler Jr, G.; Baron, J. Dexamethasone increases growth hormone receptor messenger ribonucleic acid levels in liver and growth plate. *Endocrinology* **1994**, *135*, 1113-1118.

Chapter 3: Effect of dexamethasone and route of administration on sow farrowing behaviours, piglet delivery and litter performance.

Statement of Authorship

Title of Paper	Effect of dexamethasone and route of administration on sow farrowing behaviours, piglet delivery and litter performance.
Publication Status	[x] Published [ ] Accepted for publication [ ] Submitted for publication [ ] Unpublished and Unsubmitted work written in manuscript style
Publication Details	Ward, S. A., Kirkwood, R. N., Song, Y., Garg, S., & Plush, K. J. (2022). Effect of Dexamethasone and Route of Administration on Sow Farrowing Behaviours, Piglet Delivery and Litter Performance. *Animals*, *12*(7), 847.

Principal Author

Name of Principal Author (Candidate)	Sophia Ward		
Contribution to the Paper	Designed experimental methodology, carried out in vitro and in vivo experimental procedures, analyses statistics, wrote first manuscript.		
Overall percentage (%)	70 %		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	22/02/2022

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Roy Kirkwood		
Contribution to the Paper	Conception of the theme, assisted with design of experimental methodology and drafting of the manuscript (10%)		
Signature		Date	22/02/22
Name of Co-Author			
Contribution to the Paper	Assisted with statistical analysis, drafting and evaluation of manuscript (6%)		
Signature		Date	22/02/22

Name of Co-Author	Yunmei Song		
Contribution to the Paper	Assisted and supervised in vitro investigations: preparation of ex vivo vaginal porcine tissue and Franz cell testing (8%)		
Signature		Date	22/02/22
Name of Co-Author	Sanjay Garg		
Contribution to the Paper	Assisted with drafting and evaluation of manuscript (6%)		
Signature		Date	22/02/22

animals

MDPI

Article

Effect of dexamethasone and route of administration on sow farrowing behaviours, piglet delivery and litter performance.

Sophia A. Ward[1]*, Roy N. Kirkwood[1], Yunmei Song[2], Sanjay Garg[2], and Kate J. Plush[3]

[1] School of Animal and Veterinary Sciences, University of Adelaide, Roseworthy, SA 5371, Australia; roy.kirkwood@adelaide.edu.au
[2] Clinical and Health Sciences, University of South Australia, Adelaide, SA 5000, Australia; may.song@unisa.edu.au (Y.S.); sanjay.garg@unisa.edu.au (S.G.)
[3] Sunpork Group, 1/6 Eagleview Place, Eagle Farm, QLD 4009, Australia; kate.plush@sunporkfarms.com.au
* Correspondence: sophia.ward@adelaide.edu.au

Simple Summary: The pain experienced during labor is one that is shared universally. When sows experience the pain of labor for the first time, the levels of discomfort can be so stressful that they lash out aggressively at their piglets. Sows new to the birthing experience may also have problems with delivery or resist nursing the litter for extended periods of time. To help younger sows during and after delivery, we treated a group with dexamethasone, a strong anti-inflammatory treatment. It was predicted that this anti-inflammatory would be able to provide some relief from the inflammatory pain associated with labor and help younger sows with their birthing processes and nursing of their litter. As a hormone that can easily pass through cell walls, it was also predicted that dexamethasone could pass directly through the vaginal membrane of a sow for a non-injectable treatment alternative.

Abstract: The inflammatory pain and stress some crated sows experience during farrowing has attendant risks of piglet-directed aggression, reduced teat exposure and hindered post-partum recovery. To counter this, the steroidal anti-inflammatory compound, dexamethasone, can be administered. To measure the potential for mucosal absorption as an alternative to injection, the permeability of porcine vaginal mucosa to dexamethasone was demonstrated using Franz cell diffusion. These studies found dexamethasone treatment diffused through vaginal mucosa at a constant rate, with 52.37 ± 5.54% permeation in 6 h. To examine in vivo effects on farrowing outcomes, dexamethasone was administered to gilts and parity one sows on the day of expected farrowing. We hypothesized that it would provide relief from farrowing discomfort

and reduce behaviours threatening piglet survival. Sows were randomly assigned to receive dexamethasone as an intramuscular injection (n = 23); dexamethasone applied topically into the vagina (n = 20), or to receive no dexamethasone (n = 23). Sows (n = 66) and piglets (n = 593) were monitored for performance indicators during farrowing and early lactation. A subset of sows (n = 24) was also video monitored continuously over 24 h for behaviours associated with pain, postural changes and piglet interactions. No differences were observed between treatment for farrowing performance, piglet survival or behavioural changes for sows experiencing their first or second farrowing ($p > 0.05$), rejecting the hypothesis that corticosteroid administration will improve sow farrowing performance. This investigation did, however, show that dexamethasone can permeate through porcine vaginal mucosa and so can be administered as a non-injectable treatment.

Keywords: dexamethasone; farrowing; sow behaviour; piglet performance

---

1. Introduction

Giving birth can be a stressful and painful event for sows [1], with first-time farrowings being particularly problematic. Primiparous sows tend to be more restless in farrowing crates [2] and susceptible to piglet-directed aggression [1–5]. Sows savaging piglets may not necessarily reflect poor maternal ability, but rather a nervous reaction to the pain of farrowing [5,6] in a restricting, crated environment [3]. Previous reports on sow savaging found a correlation with more restless behaviours leading up to the expulsion of the first piglet [4], which is a reportedly painful stage of parturition [1].

Farrowing behaviour and analgesic use have been studied previously [7–9] with minimal effect on sow performance or subsequent piglet survival. Unlike previously used anti-inflammatories, the use of a steroidal anti-inflammatory may be more effective for targeting relief across multiple sites of inflammatory tissue. Dexamethasone is a synthetic glucocorticoid with potent anti-inflammatory properties [10]. The potency and multi-targeted action of this drug may provide greater relief compared to other treatments and reduce aggressive behaviour exhibited by gilts (P0) and first-parity (P1) sows. Previous investigations into the use of anti-inflammatories [7–9] report administering treatment at the onset of farrowing or immediately after, once the sow

has already experienced the pain of piglet expulsion [1]. If the timing of farrowing was controlled, the anti-inflammatory could be administered in the hours leading up to parturition and may reduce the incidence of stress-induced piglet-directed aggression. Because of dexamethasone's prolonged biological half-life (36–72 h) [11], the analgesic effects may last beyond parturition and into early lactation. Reducing discomfort may encourage the sow to lie in the same position rather than making many postural changes [7,8,12], increasing teat exposure and reducing the incidence of piglet overlay. A previous investigation into the use of dexamethasone prior to farrowing found a small improvement to piglet daily gain when gilts were treated on the day of an induced farrowing [13]. By observing farrowing and early lactation behaviour, it can be determined what effect, if any, dexamethasone has on the periparturient and early lactation sow behaviours that could subsequently benefit piglet survival and growth.

A concern with using dexamethasone to relieve discomfort is the need for intramuscular injection. Injecting a young sow in the hours leading up to parturition may trigger a stress response, possibly nullifying potential benefits provided by the analgesic. As a steroid hormone, dexamethasone could potentially enter the bloodstream by diffusing through the vaginal mucosa, thus removing the need for injection. A Franz cell diffusion test can be used to measure the permeability of the vaginal mucosa and so assess the potential bioavailability of the drug when administered by this route [14]. It was hypothesised that dexamethasone would cross the vaginal mucosa and have an in vivo effect on sow behaviours and/or piglet neonatal survival.

2. Materials and Methods

2.1. Permeation of dexamethasone through porcine vaginal mucosa

To assess vaginal permeability in vitro, simulated vaginal fluid (SPVF) was prepared using the composition reported by Owen and Katz for humans [15] and adjusted with NaOH to pH 7 [16,17] to simulate conditions in the sow vagina [18,19]. Porcine vaginal tissue was obtained from a local abattoir (Murray Bridge, SA, Australia) at slaughter and transported to the laboratory in SPVF on ice. The vaginal mucosa was rinsed three times with saline, stripped from underlying connective tissue and muscle and stored at −20 °C in aluminum foil for future use. When required, the vaginal mucosa was hydrated in SPVF at room temperature and mounted onto a Franz diffusion cell.

Then, 2.5 mg (500 µL) of aqueous dexamethasone sodium phosphate treatment (Dexapent™, Troy Laboratories, Glendenning, NSW, Australia) and 200 µL SPVF was added to the mucosal surface in the donor chamber of the Franz cell. Water was heated in a water bath to 37 °C ± 1 °C and pumped around the receptor chamber. The acceptor solution (filtered SPVF) was maintained at 37 °C ± 1 °C and mixed with a magnetic stirrer throughout the experiment. Franz cell chambers (PermeGear, Hellertown, PA, USA) were set up similar to the schematic in Figure 1.

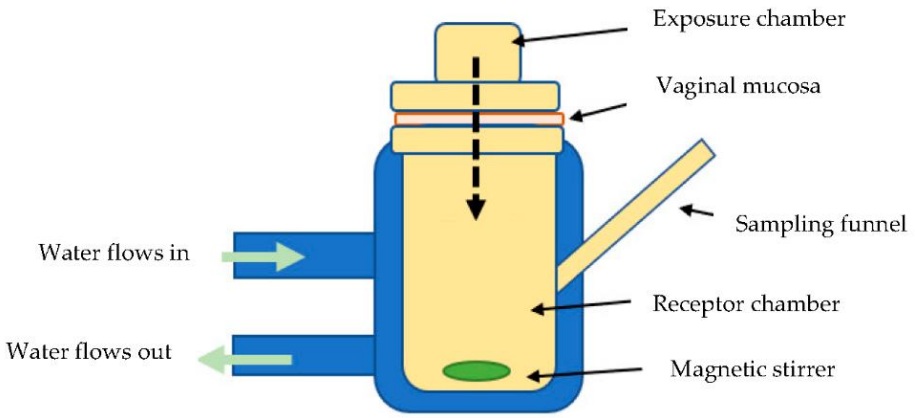


Figure 1. Schematic diagram of Franz diffusion cell.

At designated time points (0.25, 0.5, 1, 1.5, 2, 3, 4, 5 and 6 h), 100 µL of receptor fluid (SPVF) was sampled from the sampling funnel and replaced with an equal volume of fresh SPVF. All samples were prepared for liquid chromatography by adding 50 µL samples to 50 µL mobile phase and vortexed before HPLC analysis.

The separation system consisted of a Lux Cellulose-1 column (Phenomenex Australia, Lane Cove, NSW, Australia) and 1% formic acid in acetonitrile (solvent A) and 2% formic acid in water (solvent B) in a 50:50 ratio. The method was based on the isocratic method used by Karatt et al. [20] with mobile phase A acidified to obtain sharper peak resolution. Flow rates were set at 0.6 mL/min with the column temperature at 50 °C and detected using a wavelength of 241 nm. Calibration graphs were constructed by plotting the peak area with their corresponding concentrations of dexamethasone (linearity range: 2.5–300

ug/mL). The sum of the two observable peaks not present in blank SVF was calculated with $r^2 = 0.99$ (Figure 2).

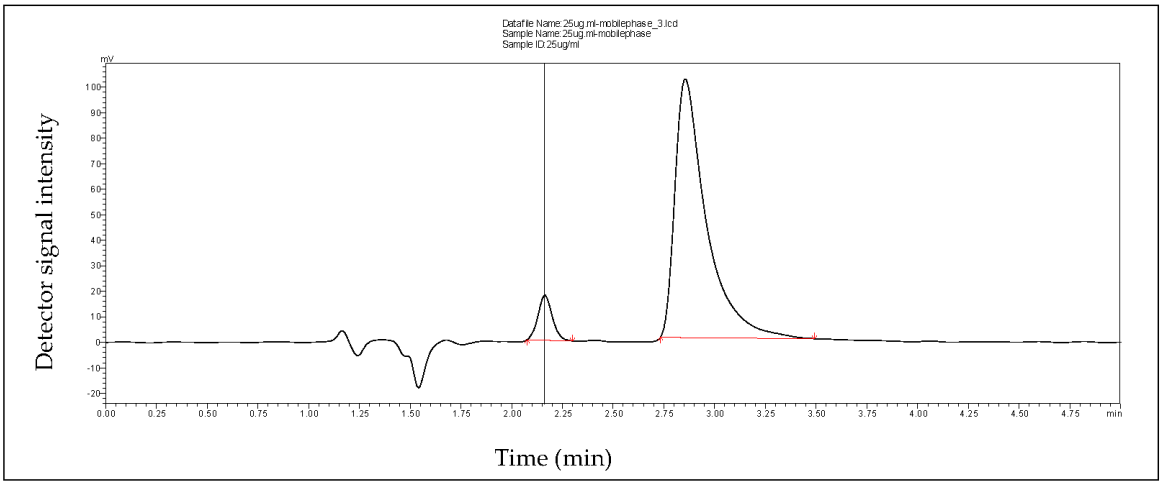


Figure 2. Chromatogram of dexamethasone treatment.

Permeability was calculated using the following formula:

$$x = ((TsV)/OC) \times 100\%$$

Where:
x = Cumulative amount of drug through vaginal mucosa (%)
V = Total volume in Franz cell (5.0 mL)
OC = Amount of drug administered in donor compartment (2.5 mg)
Ts = Concentration of the sample taken from Franz cell acceptor solution

Release parameters for the permeation of dexamethasone through porcine vaginal mucosa were calculated using the analysis program created by Zhang et al. [21].

2.2. Effects of Dexamethasone on Sow and Piglet Performance

2.2.1. Animal Management

Large White × Landrace gilts and P1 sows (sows) were moved into individual farrowing crates one week before their expected due dates of 116 d after the last insemination. Gilts and sows were fed twice daily with a commercial diet formulated to meet all nutrient requirements and had free access to fresh water. At 114 d of gestation, sows received vulva injections of 125 µg prostaglandin analogue, cloprostenol (Juramate®, Jurox Pty, Ltd., Rutherford, NSW, Australia), at 0700 and 1300 h to induce sows to farrow on day 115 of gestation. At 0800 h on day 115, sows were randomly assigned to receive 20 mg dexamethasone (Dexapent™, Troy Laboratories, Glendenning, NSW, Australia) either by intramuscular injection (n = 23; DexInj), by topical vaginal mucosal deposition (n = 20; DexTop) or to serve as non-treated controls (n = 23) with equal parity distribution. To administer the DexTop treatment, a thin sterile

tube was inserted 20 cm into the vagina, and treatment was administered followed by a 0.5 mL saline flush.

2.2.2. Data Collection

Farrowing duration, total born litter size, stillbirths, incidence of dystocia and piglet overlay in the first 24 h postpartum were recorded as indicators of sow performance. If the piglet delivery interval exceeded 45 min, obstetric assistance was provided for sows, and it was recorded as a dystocia event. The estimated colostrum intake of piglets was calculated using their birth and 24 h weights and the equation proposed by Devillers et al. [22].

$$CI = -217.4 + 0.217 \times t + 1861019 \times BW/t + BWb \times (54.8 - 1861019/t) \times (0.9985 - 3.7 \times 10 - 4 \times tfs + 6.1 \times 10 - 7 \times t^2fs)$$

where CI = colostrum intake (g), BWb = piglet body weight at birth, BW = piglet body weight at 24 h and t = time elapsed from birth to first suckling (min).

Devillers et al. [22] proposed that the interval of elapsed time from birth to first suckling can be estimated as between 15 and 30 min without major error. In our study, the average interval was 20 min.

2.2.3. Farrowing Behaviour

A subset of sows was video recorded for 24 h from the onset of farrowing using CCTV cameras mounted above each farrowing crate. Sows were assigned either to the DexInj (n = 8), DexTop (n = 9) or Control (n = 9) treatment group. Continuous state and point behaviour observations were made by one observer using the ethogram program BORIS. Potential behavioural indicators of pain were based on Ison et al. [23] (Table 1).

Table 1. Ethogram used for monitoring farrowing behaviour over a 24 h period.

	Behaviour	**Description**
Posture	Stand	Sow is standing.
	Sit	Sow is sitting
	Side lie	Lateral recumbency: udder or at least the top line or teats are not obscured.
	Belly lie	Sternal recumbency: the udder is obscured under the sow.

Spontaneous behaviours	Tail flick	The tail is moved rapidly up and down.
	Back leg forward	In a lateral lying position, the back leg is pulled forwards and/or in towards the body.
	Back arch	In a lateral lying position, one or both sets of legs become tense and are pushed away from the body and in towards the center, forming an arch in the back.
	Paw	The sow uses the forepaw to scrape the floor in a pawing position.
	Piglet-directed aggression	The sow snout flicks quickly behind/snaps at the approaching piglet.
	Overlay	Any event where a piglet is being crushed by the sow. Piglets may be under the sow, squashed at the front of the crate or under the trotter.

2.2.4. Statistics

Data were analyzed using IBM SPSS v20 statistical software. For the primary outcome, effects of dexamethasone on observational behavior, data were analyzed using a general linear model with a negative binomial distribution. The binary measurements in this investigation (incidence of stillbirth, dystocia, overlay and piglet-directed aggression) were assessed using a generalized linear model fit with binomial distribution. Other outcomes of interest (farrowing duration, piglet birth interval, total piglets born, total piglets born alive and litter size weaned) were measured with a linear mixed model. All data pertaining to sows was fit with treatment (DexInj, DexTop or Control) and parity (gilt or first-parity sow) as fixed effects. The model included sow ID and room (identical farrowing rooms 4 and 5) as random effects.

For measurements pertaining to the piglet (colostrum intake, survival of piglets to 24 h and piglet survival to weaning), outcomes were assessed using a general linear model with sow as the subject and birth order as the repeated measure. Fixed effects included sow treatment (DexInj, DexTop or Control), piglet gender (male/female) and birth weight group (<1.0 kg, low; 1.1–1.35 kg, medium; >1.35 kg, heavy) All data in the investigation were analysed with a confidence limit set at 95% ($p < 0.05$).

3. Results

In Vitro Permeability of Dexamethasone through the Vaginal Mucosa

Over the 6 h of the Franz cell test, dexamethasone passed through the vaginal mucosa in an increasing linear function (Figure 3).

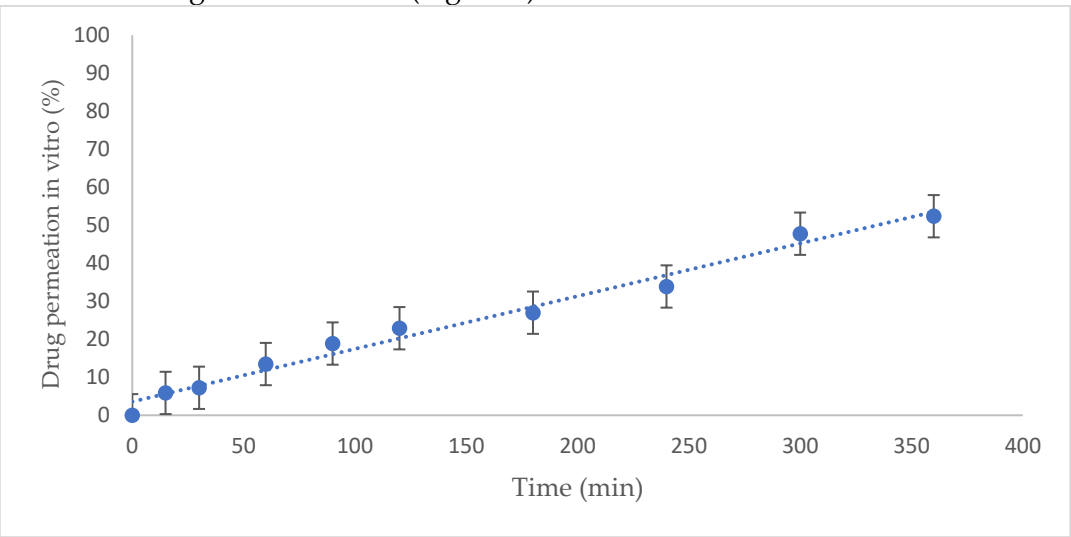


Figure 3. In vitro permeation of dexamethasone treatment (%) through porcine vaginal mucosa using Franz diffusion cells over time (minutes). Simulated vaginal fluid was used for the donor and acceptor solutions, and cells were incubated at 37 °C ± 1·°C. The diffusion tests were run six times and are presented as the mean ± standard deviation (SD) of the mean.

The rate at which dexamethasone diffused across the vaginal mucosal membrane is best described by Makoid–Banakar, with an r^2 = 0.9851 and a magnitude of data or AIC = 33.67 (Figure 4, Appendix A).

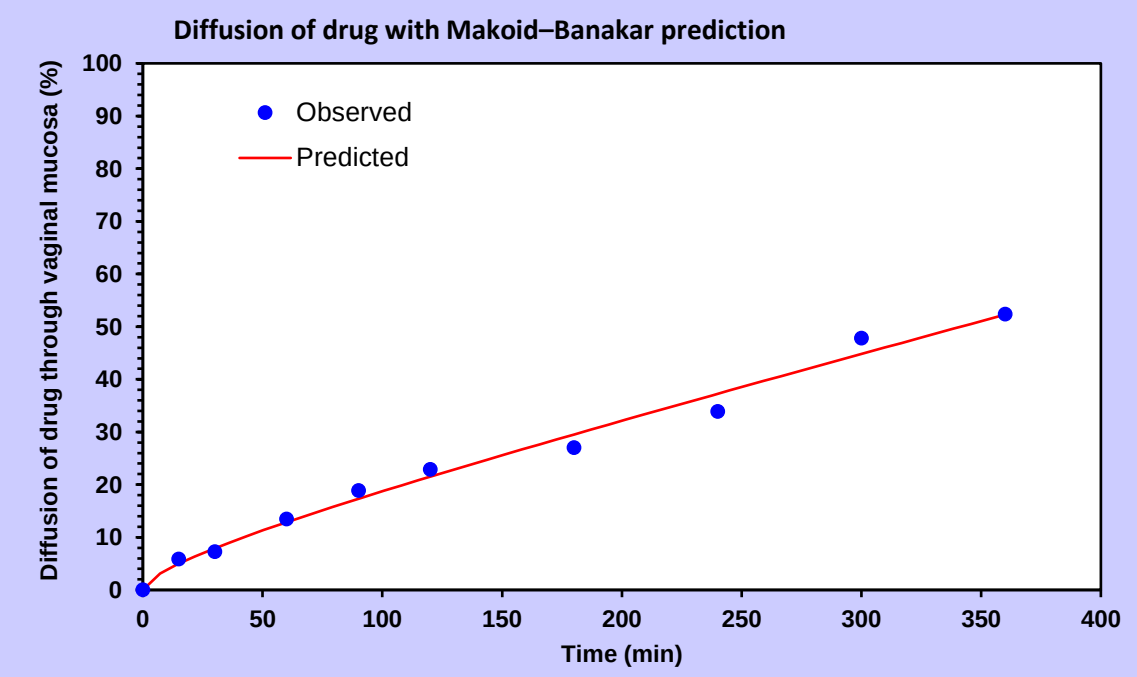


Figure 4. A diffusion profile of dexamethasone treatment through porcine vaginal mucosa in-vitro fitted by Makoid–Banakar model (F = kMB × t^n × Exp(−k × t). r^2 adjusted = 0.9871 and AIC =39.2.

3.2. Farrowing performance parameters.

As shown in Table 2, treatment had no significant effect on farrowing performance, with no differences in the duration of farrowing ($p = 0.214$), piglet birth interval ($p = 0.289$) or stillbirths ($p = 0.655$), although gilts had comparatively shorter birth intervals compared to P1 sows ($p = 0.006$; Gilts = 11.53 ± 1.3 min; Sows = 18.06 ± 1.9 min). There was also a trend towards higher incidence of dystocia for P1 sows compared to gilts ($p = 0.068$; Gilts = 28 ± 7%; P1 = 54 ± 12%), but no differences between treatment groups was evident ($p = 0.263$). No treatment effects were observed for the colostrum intake of piglets ($p = 0.718$), but differences in intake were observed across the three piglet birthweight groups, with intake increasing with increasing body weight (BW) ($p = 0.001$; Low BW = 278.1 ± 7.0 g; Medium BW = 326.2 ± 4.9 g; Large BW = 349.4 ± 5.0 g). The provision of dexamethasone had no effect on incidence of overlay ($p = 0.393$) or piglet survival in the first 24 h ($p = 0.872$), although a trend was observed for survival to weaning ($p = 0.094$) (Table 2).

Table 2. Effects of dexamethasone administered the day of farrowing (0700 h, gestation day 115) as a vulval injection (DexInj), applied topically into the vagina (DexTop), or no treatment (Control) on mean (±SE) sow and piglet performance indicators.

	Control	**DexInj**	**DexTop**	***p* Value**
Farrowing duration (min)	232 ± 26	157 ± 42	170 ± 31	0.214
Piglet birth interval (min)	17.1 ± 1.8	13.2 ± 1.7	14.1 ± 1.7	0.289
Incidence of dystocia (%)	47 ± 11	21 ± 8	42 ± 11	0.263
Incidence of stillbirth (%)	67 ± 10	68 ± 9	57 ± 11	0.655
Total piglets born	11.7 ± 0.5	11.7 ± 0.5	11.8 ± 0.3	0.943
Total piglets born alive	11.3 ± 0.6	11.1 ± 0.6	11.0 ± 0.6	0.952
Colostrum intake (g)	319.3 ± 6.7	313.5 ± 6.5	320.3 ± 6.5	0.718
Incidence of overlay in 24 h (%)	57 ± 11	39 ± 10	57 ± 11	0.393
Piglet survival to 24 h (%)	89.6 ± 2.8	91.5 ± 2.7	90.9 ± 2.8	0.872
Litter size weaned	10.4 ± 0.3	10.6 ± 0.3	10.6 ± 0.3	0.855
Survival of piglets to weaning (%)	79.9 ± 2.9	88.8 ± 2.9	86.6 ± 3.0	0.094

Over the 24 h observational period, no differences were observed in individual or total pain behaviours among treatments ($p > 0.05$; Table 3). The incidence of piglet-

directed aggression and time spent on the side were all similar between treatment groups.

Table 3. Effects of dexamethasone administered the day of farrowing (0700 h, gestation day 115) as a vulval injection (DexInj), applied topically into the vagina (DexTop), or no steroid treatment (Control) on mean (±SE) sow behaviours during 24 h from onset of farrowing. The number of sows that displayed any piglet-directed aggression is expressed as a percentage over the total treatment group (95% CI). In addition, the time each sow spent in the laying position (udder exposed) is presented as a percentage over the total 24 h observation period.

	Control	DexInj	DexTop	*p* Value
Back arch	9.9 ± 3.7	4.1 ± 1.6	5.8 ± 2.4	0.256
Leg up	12.3 ± 4.9	4.3 ± 1.8	7.6 ± 2.9	0.199
Pawing	8.8 ± 3.2	6.0 ± 2.5	3.4 ± 1.5	0.321
Tail flick	0.1 ± 0.1	1.2 ± 0.5	0.4 ± 0.1	0.088
Total pain behaviours	22.5 ± 4.6	15.5 ± 4.9	19.0 ± 4.6	0.525
Total position changes	77.3 ± 11.8	53.8 ± 11.8	69.3 ± 12.3	0.382
Piglet-directed aggression (%)	50 ± 5	25 ± 4	22 ± 4	0.269
Time spent on side (%)	81.6 ± 3.6	87.8 ± 3.6	82.8 ± 3.8	0.350

4. Discussion

4.1. Franz Cell Permeation Test

Within 6 h, half of the dexamethasone passed through the sow vaginal mucosa, closely following Makoid–Banakar release model kinetics. As predicted, the lipophilic properties of the steroid enabled rapid diffusion across the vaginal mucosal membrane. This rate of diffusion in the present study was slower than permeability reported by Zang et al. [24], who found 60% of dexamethasone sodium phosphate in a film passed through rabbit buccal mucosa within the first 2.5 h. Differences between release studies could be due to differences in an animal model (sow vs. rabbit), mucosa type (vaginal vs. buccal) and/or properties of drug delivery formulation (injectable solution vs. buccal film). With evidence of permeation, future investigations should track the concentration of dexamethasone in sow plasma over time, measuring the concentration with HPLC–mass spectrometry.

Although the treatment used in our study is a dexamethasone product, the two definite peaks present in chromatographs suggest possible traces of another active constituent within the formulation [25]. When Xiao et al. [25] tested a solution with 1%

betamethasone in pure dexamethasone, small and large peaks presented on the chromatographs. These peaks increased with increasing concentrations of the solution, similar to what was found with the chromatograph in our study. Bentamethasone is a chemical isomer with similar anti-inflammatory properties to dexamethasone [25].With this considered, the permeation rate of dexamethasone through the vaginal mucosa cannot be definitively defined without clarifying these two peaks against pure betamethasone standard reference. Our chromatographs did give evidence for the passage of dexamethasone through porcine vaginal mucosa, which concurs with our initial hypothesis.

4.2. Animal Treatment

With the confirmation of dexamethasone permeating through porcine vaginal mucosa in vitro, we tested it in vivo by deposition onto sow vaginal mucosa. Our data did not show evidence of dexamethasone affecting sow performance or farrowing behaviours. These results are similar to studies using non-steroidal anti-inflammatory (NSAIDs) meloxicam [8], and analgesic butorphanol [7] on post-partum sows. Mainau et al. [8] proposed that the lack of NSAID effect on farrowing performance was the result of administering treatment too late, but treatment in our study was administered before the expulsive phase of farrowing. Our data would suggest the lack of treatment effect on sow behaviour was not the result of administration before or after farrowing onset. Further, the potency of the anti-inflammatory agent used may also not be a critical factor in changing sow performance, as the use of the potent analgesic, butorphanol showed no differences to pain related behaviours within the first 48 h postpartum [7]. What was observed was a significant reduction in posture changes during the 48 h after treatment, which may have reduced the risk of piglet crushing during the nursing period. This may explain why a trend was observed in our data for improved piglet survival for dexamethasone-treated sows.

Lay et al. [26] suggested that anti-inflammatory compounds have little effect on the farrowing sow due to the restrictive nature of farrowing crates. An increase in the nociceptive threshold is mediated by endogenous opioids, which can be inhibited when a sow is restricted from maternal behaviours leading up to parturition [1]. Nowland et al. [27] observed fewer pain-related behaviours (tail flicking, back leg forward, and straining) when sows were housed in open pens over traditional crates. In another

investigation, Nowland et al. [28] found less pain-related behaviours in crated sows when they had access to straw in the lead up to farrowing (n = 12) [29]. Although the number of sows used for the behavioral studies was similar to those reported by Nowland et al. [28], a larger sample size of predominately primiparous sows should increase the chances of observing restless behaviours, aggressive tendencies, or animals with a higher susceptibility to pain during parturition. Why the provision of an anti-inflammatory does not provide the same analgesic response is something that should be assessed further, particularly if it is coupled with an improvement in farrowing performance. The use of anti-inflammatories may be more beneficial in sow herds that have higher preweaning mortality rates [29] or pre-existing conditions where analgesia would alleviate discomfort [30,31]. Additionally, the effects of dexamethasone may be more evident in larger populations, as the levels of discomfort experienced during parturition can be subjective to the individual [1].

5. Conclusions

Administering dexamethasone on the day of an induced farrowing did not affect sow behaviours during parturition and early lactation. This would imply that some level of pain and/or discomfort is normal during parturition and the immediate post-partum period and, as such, would not be responsive to anti-inflammatory treatments. It is possible that a beneficial effect of steroid would be evident only under conditions of abnormal levels of pain or distress, as potentially indicated by elevated levels of pre-weaning mortality.

Author Contributions: Conceptualization, R.K and S.W; methodology, R.K, S.W and Y.S; investigation, S.W, writing-original draft preparation, S.W; writing-review and editing, R.K, K.P, S.G and Y.S; supervision, R.K, K.P, S.G and Y.S. All authors have read and agreed to the published version of this manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The University of Adelaide Animal Ethics Committee approved this study (S-2020-006).

Informed Consent Statement: Not Applicable

Data Availability Statement: The data presented in this study are available on request from the corresponding author

Acknowledgments: The authors would like to thank the Pharmaceutical Innovation and Development Group (PIDG) at the University of South Australia for their guidance and access to equipment for in vitro testing, the workers at the University of Adelaide Roseworthy Piggery for care and maintenance of animals and the Murray Bridge Abattoir for access to porcine vaginal

tissue samples. Sophia also gratefully acknowledges the University of Adelaide and Australian Pork Limited for funding her research scholarship.

Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

Table A1. Release parameters of fitted experimental data for in vitro permeation of dexamethasone through porcine vaginal mucosa. The best fit model will have a closest coefficient value to 1.0 (r^2 adjusted) and a lower magnitude of data (AIC). Highlighted is the model that was deemed the best fit for the data.

Model Name	Equation	Goodness of Fit Parameter	Value
Zero order	$F = k_0 \times t$	r^2 adjusted	0.9627
		AIC	48.53
First order	$F = 100 \times (1 - \mathrm{Exp}(-k_1 \times t))$	r^2 adjusted	0.9785
		AIC	42.92
Higuchi	$F = k_H \times t^{0.5}$	r^2 adjusted	0.9206
		AIC	56.00
Hixson–Crowell	$F = 100 \times (1 - (1 - kHC \times t)^3)$	r^2 adjusted	0.9766
		AIC	43.80
Hopfenberg	$F = 100 \times (1 - (1 - kHB \times t)^n)$	r^2 adjusted	0.9758
		AIC	44.93
Makoid–Banakar	$F = kMB \times t^n \times \mathrm{Exp}(-k \times t)$	r^2 adjusted	0.9871
		AIC	39.29
Baker–Lonsdale	$3/2 \times (1 - (1 - F/100)^{2/3}) - F/100 = k\mathrm{BL} \times t$	r^2 adjusted	0.8977
		AIC	58.53
Peppas–Sahlin	$F = k_1 \times t^m + k_2 \times t^{2m}$	r^2 adjusted	0.9850
		AIC	40.80

References

1. Mainau, E.; Manteca, X. Pain and discomfort caused by parturition in cows and sows. *Appl. Anim. Behav. Sci.* **2011**, *135*, 241–251.
2. Harris, M.; Li, Y.; Gonyou, H. Savaging behaviour in gilts and sows. *Can. J. Anim. Sci.* **2003**, *83*, 819–821.
3. Ahlström, S.; Jarvis, S.; Lawrence, A. Savaging gilts are more restless and more responsive to piglets during the expulsive phase of parturition. *Appl. Anim. Behav. Sci.* **2002**, *76*, 83–91.
4. Chen, C.; Gilbert, C.L.; Yang, G.; Guo, Y.; Segonds-Pichon, A.; Ma, J.; Evans, G.; Brenig, B.; Sargent, C.; Affara, N. Maternal infanticide in sows: Incidence and behavioural comparisons between savaging and non-savaging sows at parturition. *Appl. Anim. Behav. Sci.* **2008**, *109*, 238–248.
5. Dove, C.R. Farrowing and Lactation of the Sow and Gilt. *Livest. Prod. Sci.* **2009**, *66*.
6. Peltoniemi, O.; Oliviero, C. Housing, management and environment during farrowing and early lactation. In *The Gestating and Lactating Sow*; Wageningen Academic Publishers: Wageningen, The Netherlands, 2015; pp. 77–84.
7. Haussmann, M.; Lay, D.; Buchanan, H.; Hopper, J. Pain Relief in Sows Can Help Reduce Post-Farrowing Activity, Which Could Lead to Reduced Piglet Crushing. *Iowa State Univ. Anim. Ind. Rep.* **1999**, *1*.
8. Mainau, E.; Ruiz-De-La-Torre, J.; Dalmau, A.; Salleras, J.; Manteca, X. Effects of meloxicam (Metacam®) on post-farrowing sow behaviour and piglet performance. *Animal* **2012**, *6*, 494–501.
9. Ison, S.H.; Jarvis, S.; Ashworth, C.J.; Rutherford, K.M. The effect of post-farrowing ketoprofen on sow feed intake, nursing behaviour and piglet performance. *Livest. Sci.* **2017**, *202*, 115–123.
10. Barnes, P.J. How corticosteroids control inflammation: Quintiles prize lecture 2005. *Br. J. Pharmacol.* **2006**, *148*, 245–254.
11. Shefrin, A.E.; Goldman, R.D. Use of dexamethasone and prednisone in acute asthma exacerbations in pediatric patients. *Can. Fam. Physician* **2009**, *55*, 704.
12. Tenbergen, R.; Friendship, R.; Cassar, G.; Amezcua, M.; Haley, D. Investigation of the use of meloxicam for reducing pain associated with castration and tail docking and improving performance in piglets. *J. Swine Health Prod.* **2014**, *22*, 64–70.
13. Ward, S.A.; Kirkwood, R.N.; Plush, K.J. Are larger litters a concern for piglet survival or an effectively manageable trait? *Animal* **2020**, *10*, 309.
14. Machado, R.M.; Palmeira-de-Oliveira, A.; Gaspar, C.; Martinez-de-Oliveira, J.; Palmeira-de-Oliveira, R. Studies and methodologies on vaginal drug permeation. *Adv. Drug Deliv. Rev.* **2015**, *92*, 14–26.
15. Owen, D.H.; Katz, D.F. A vaginal fluid simulant. *Contraception* **1999**, *59*, 91–95.
16. Borges, S.F.; Silva, J.G.; Teixeira, P.C. Survival and biofilm formation of Listeria monocytogenes in simulated vaginal fluid: Influence of pH and strain origin. *FEMS Immunol. Med. Mircobiol.* **2011**, *62*, 315–320.
17. Machado, R.M.; Tomás, M.; Palmeira-de-Oliveira, A.; Martinez-de-Oliveira, J.; Palmeira-de-Oliveira, R. The vaginal sheet: An innovative form of vaginal film for the treatment of vaginal infections. *Drug Dev. Ind. Pharm.* **2020**, *46*, 135–145.
18. Lorenzen, E.; Follmann, F.; Jungersen, G.; Agerholm, J.S. A review of the human vs. porcine female genital tract and associated immune system in the perspective of using minipigs as a model of human genital Chlamydia infection. *Vet. Res.* **2015**, *46*, 116.
19. López-Albors, O.; Llamas-López, P.J.; Ortuño, J.Á.; Latorre, R.; García-Vázquez, F.A. In vivo measurement of pH and CO_2 levels in the uterus of sows through the estrous cycle and after insemination. *Sci. Rep.* **2021**, *11*, 1–11.
20. Karatt, T.K.; Sayed, R.; Nalakath, J.; Perwad, Z.; Albert, P.H.; Khader, K.A. Separation and identification of the epimeric doping agents–Dexamethasone and betamethasone in equine urine and plasma: A reversed phase chiral chromatographic approach. *Steroids* **2018**, *140*, 77–82.
21. Zhang, Y.; Huo, M.; Zhou, J.; Zou, A.; Li, W.; Yao, C.; Xie, S. DDSolver: An add-in program for modeling and comparison of drug dissolution profiles. *AAPS J.* **2010**, *12*, 263–271.
22. Devillers, N.; van Milgen, J.; Prunier, A.; Le Dividich, J. Estimation of colostrum intake in the neonatal pig. *Anim. Sci.* **2004**, *78*, 305–313.
23. Ison, S.H.; Jarvis, S.; Rutherford, K.M. The identification of potential behavioural indicators of pain in periparturient sows. *Res. Vet. Sci.* **2016**, *109*, 114–120.
24. Zhang, C.; Liu, Y.; Li, W.; Gao, P.; Xiang, D.; Ren, X.; Liu, D. Mucoadhesive buccal film containing ornidazole and dexamethasone for oral ulcers: In vitro and in vivo studies. *Pharm. Dev. Technol.* **2019**, *24*, 118–126.
25. Xiao, K.P.; Xiong, Y.; Rustum, A.M. Quantitation of trace betamethasone or dexamethasone in dexamethasone or betamethasone active pharmaceutical ingredients by reversed-phase high-performance liquid chromatography. *J. Chromatogr. Sci.* **2008**, *46*, 15–22.
26. Lay, D.; Haussmann, M. Effect of Ibuprofen Administration on Postural Changes in Post-Parturient Sows. *Iowa State Univ. Anim. Ind. Rep.* **2000**, *1*.

27. Nowland, T.; van Wettere, W.; Plush, K. Confinement of sows at parturition increases the incidence of behaviours thought to indicate pain. *Anim. Prod. Sci.* **2017**, *57*, 2444–2444.
28. Nowland, T.L.; van Wettere, W.H.E.J.; Plush, K.J. Allowing sows to farrow unconfined has positive implications for sow and piglet welfare. *Appl. Anim. Behav. Sci.* **2019**, *221*, 104872.
29. Homedes, J.; Salichs, M.; Sabaté, D.; Sust, M.; Fabre, R. Effect of ketoprofen on pre-weaning piglet mortality on commercial farms. *Vet. J.* **2014**, *201*, 435–437.
30. Tummaruk, P.; Sang-Gassanee, K. Effect of farrowing duration, parity number and the type of anti-inflammatory drug on postparturient disorders in sows: A clinical study. *Trop. Anim. Health Prod.* **2013**, *45*, 1071–1077.
31. Revilla, E.; Crisol, M.; Lising, R.; Salleras, J. Effect of Treatment with Metacam® in sows with MMA syndrome on the viability and growth of piglets born with a low birth weight. *Plenary Sess.* **2005**, *10*, 4–49.

Chapter 4: Administering dexamethasone to primiparous sows: effect of macromolecular uptake of low birthweight piglets.

1

Statement of Authorship

Title of Paper	Administering dexamethasone to primiparous sows: effect of macromolecular uptake of low birthweight piglets.
Publication Status	☐ Published ☐ Accepted for publication ☐ Submitted for publication X Unpublished and Unsubmitted work written in manuscript style
Publication Details	Submitted as an abstract to Australian Association of Animal Sciences conference

Principal Author

Name of Principal Author (Candidate)	Sophia Ward		
Contribution to the Paper	Designed experimental methodology, carried out in vitro and in vivo experimental procedures, analyses statistics, wrote first manuscript.		
Overall percentage (%)	80 %		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	22/02/2022

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Roy Kirkwood		
Contribution to the Paper	Conception of the theme, assisted with design of experimental methodology and drafting of the manuscript (10%)		
Signature		Date	22/02/22
Name of Co-Author	Kate Plush		
Contribution to the Paper	Assisted with statistical analysis, drafting and evaluation of manuscript (10%)		
Signature		Date	22/02/22

Administering glucocorticoids to primiparous sows: effects on macromolecule uptake of low birthweight piglets

Sophia A. Ward[1]*, Roy N. Kirkwood[1], Kate J. Plush[2]

[1] School of Animal and Veterinary Sciences, The University of Adelaide, SA, Australia, 5371.
[2] SunPork Group, Murarrie, QLD Australia 4172
* Correspondence: Sophia Ward, School of Animal and Veterinary Sciences, The University of Adelaide, Roseworthy SA 5371, Australia. Email: sophia.ward@student.adelaide.edu.au

Received: date; Accepted: date; Published: date

Abstract

Glucocorticoids are essential regulators of late fetal development, with effects on organ maturation being time specific. As gut maturation in the piglet occurs at the end of gestation, it was hypothesised that treating sows with dexamethasone before farrowing would accelerate enteric maturation in piglets and improve macromolecular absorption. Primiparous sows were induced to farrow by injections of cloprostenol at 0700 and 1300 h at 2 d before due date (d114) and assigned either no further treatment (Control) or an intramuscular injection of 20 mg dexamethasone at 24 h after first cloprostenol injection. Low birthweight (≤1.0kg) and normal birthweight (1.0kg - 1.3 kg) piglets were given 3 h unrestricted access with the sow before oral administration of a marker solution, FITC-D (25 mg/ml), diluted in phosphate buffered saline. Blood samples were taken at 2 h and 4 h post gavage and plasma assayed for FITC-D using fluorescence spectrometry (480 nm, 520 nm). Although treating gilts with dexamethasone pre-partum did not influence the absorption of FITC-D in progeny, there was a trend observed for piglets in smaller litters and a greater absorption of FITC-D at the fourth hour (P = 0.66). These results suggests maternal treatment of dexamethasone in late gestation does not influence uptake of macromolecules in newborn piglets, but litter size may play an important role.

Keywords: piglet; gut; newborn, glucocorticoids; dexamethasone

1. Introduction

Gilts are an important part of breeding herd [1], yet their progeny can be a serious constraint to productivity on farm [2]. Due to competition with maternal resources for growth [3] gilt progeny are susceptible to restricted development in utero [4,5] causing slower growth rates and increased mortality [1,2]. The severity of this restricted development can be assessed by head morphology [6,7] but any piglet born under 1000 grams is at risk of disease susceptibility and impaired growth [7].

Piglet growth is determined, in part, by uterine capacity and development of the placenta [8]. Whilst uterine capacity is limited, placental development can be affected by prenatal exposure to natural or synthetic glucocorticoids (GCs). GCs act as an important developmental switch [9] able to enhance (but not initiate) expression of genes in accordance with critical stages of fetal development [10,11]. Exposing the fetus to high levels of GCs during mid gestation can alter genes coding for skeletal growth and stunt birthweight [12]. In contrast, exposure to the same GCs in very late gestation can trigger maturation of specific organs in preparation for birth [9]. Reported effectiveness of GC fetal exposure and development depends on the time when the organ of interest is most sensitive to GC uptake.

The link between maternal glucocorticoid (GC) exposure and fetal organ maturation has been researched in a variety of mammalian models [13-15] but its effects on the gastrointestinal tract of piglets is less studied. In the piglet, gastrointestinal maturation occurs in the late fetal and early neonatal stages [10] which is predicted to be the time in which GC receptors in the gut are most responsive. Gut function of newborn piglets is critical for survival as piglets acquire immunity by absorbing immunoglobulins from colostrum [16,17]. Effective absorption of these macromolecules prior to gut closure will protect piglets from early onset disease and lays the microbial foundation for future survival [18].

Gut permeability in the newborn piglet is regulated by enterocyte gap junctions, and the distribution and function of these gap junctions determine the efficiency by which macromolecules can pass into circulation [19]. In the final week of gestation, sows with a low concentration of GCs gave birth to piglets with less functioning gap junctions and a lower absorption rate of IgG proteins [20]. Having high concentration of GCs before farrowing may allow for final development of gap junctions and improve intestinal permeability of neonates.

To measure gap junction distribution and function, several studies have used non-toxic molecular markers. Westrom et al [21] found Fluorescein isothiocyanate-dextran (FITC-D) could be used to assess the gut function of newborn piglets [21,22]. The level of fluorescence indicates the amount of dextran present, providing a simple and effective measurement of absorption. It is predicted that maternal gilt exposure to synthetic GCs will increase the concentration of FITC-D in the plasma of low birthweight piglets and, in turn, indicate an increased absorptive capacity for macromolecules.

2. Materials and Methods

2.1. Sow Management

Large White x Landrace gilts were moved into individual farrowing crates one week before their expected due date. Sows were fed a commercially formulated diet at 2.5 kg/d and had free access to fresh water. Two days before their expected farrowing date, gilts received vulva injections of 125μg of the prostaglandin F2α analogue, cloprostenol (Juramate®, Jurox Pty, Ltd, Rutherford, NSW, Australia; PGF), at 0700 and 1300 h. At 24 h after the first PGF injection, gilts were assigned to receive an intramuscular injection of 20 mg dexamethasone (Dex; n=7) or to act as non-injected controls (Control; n=7).

2.2. Experiment

From delivery, piglets were allowed 3 h of free access to the sow udder for consumption of colostrum. At 3 h after delivery, the two lightest piglets in the litter (0.94 ± 0.02 kg) and two normal birthweight piglets (1.32 ± 0.02 kg) were removed from each gilt and placed in a crèche under infrared heat (34°C). Using a piglet feeding tube, piglets received 10 mL/kg body weight FITC-D (4,000 Daltons, 25 mg/mL; Sigma Aldrich) dissolved in 0.9% saline. Piglets remained in the crèche until blood sampling was completed via anterior vena cava venipuncture at 2 and 4 h after receiving FITC-D. Following the 4 h bleed, the piglets were placed back on their sow. Blood samples were centrifuged for 10 min at 3000 rpm and plasma stored at -20˚C until required for FITC-D assay.

2.3 FITC-D analysis:

Samples were thawed at 4˚C prior to assay and protected from light. Plasma samples (50 μL) were diluted with 1.9 mL saline prior to plate loading. Diluted samples (60 μL) and

standards were assayed in triplicate on flat bottom black 96-well plates (Sigma Aldrich) using a Synergy MX plate reader (Biotek Instruments, Bedfordshire, UK) at an excitation wavelength of 480 nm and emission wavelength of 520 nm [21]. Concentrations of FITC-D were calculated using the formula generated by the standard curve of each plate ($R^2 \geq 0.97$). To calculate relative absorption, total concentrations (μg/mL) were converted to mg/mL, divided by administered concentration (25 mg/mL * volume given by birthweight) and expressed as a percentage of absorption. All samples were protected from light during assay and read immediately after the plate was loaded.

2.4. Statistical analysis:

All data were analysed using IBM SPSS v20. Prior to analysis, data were examined for normal distribution. The sow treatment (control; dexamethasone), piglet gender (male; female), birthweight category (low < 1.1 kg; normal >1.3 kg) and litter size (smaller ≤ 11 piglets; larger ≥ 12 piglets) were fit as fixed effects with two-way interactions fitted. Data were analysed with significance held at the 95% level of confidence and defined as statistically different when $P \leq 0.05$. Data were analysed and are presented as means ± standard error of the mean.

3. Results

The average concentration of FITC-D in piglet plasma increased over time, with maternal treatment of dexamethasone having no effect on absorption for either time points (Figure 1).

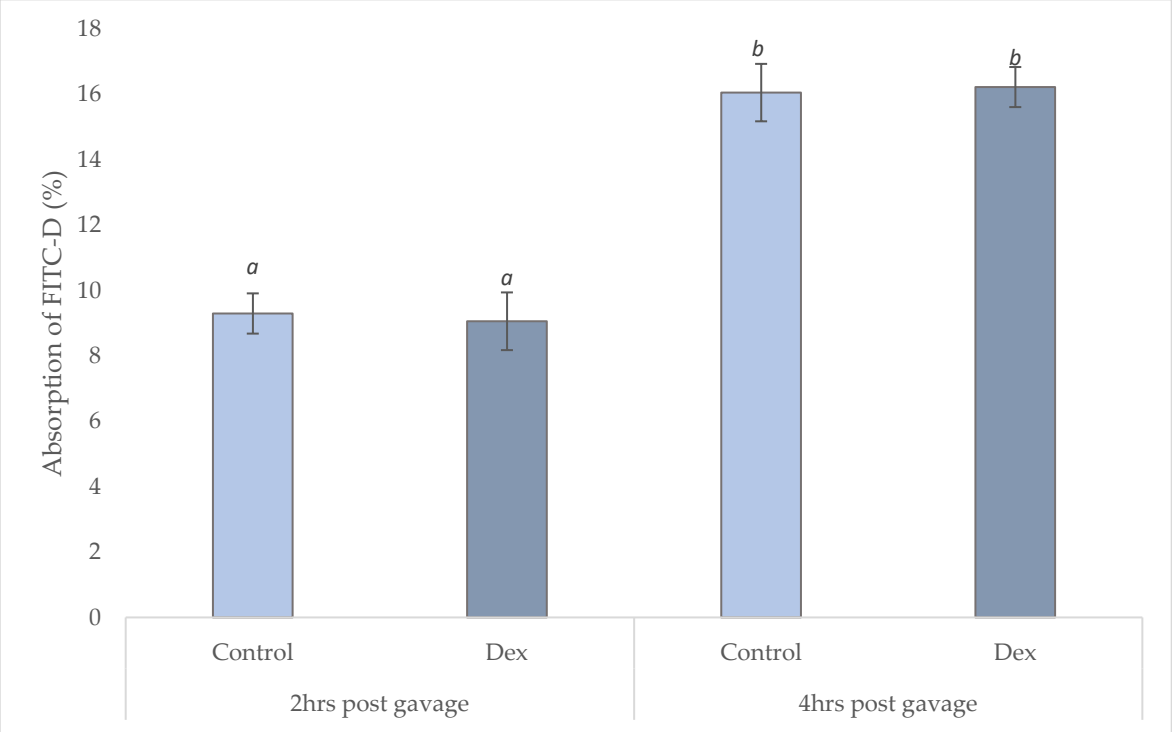


Figure 1. Effects of dexamethasone (Dex) or Control administered on the day of induced farrowing (day 115 of gestation at 7 am) on the relative absorption of FITC-D (25 mg/kg BW) in the plasma of newborn piglets 2 and 4 hrs after ingestion. Significant differences at $P < 0.05$ are represented with a and b superscripts.

No two or three-way factors affected the absorption of FITC-D, although a trend was observed between litter size and absorption at the fourth hour post gavage (P=0.066) (Table 1).

Table 1. The effect of litter size (smaller ≤ 11; larger ≥ 12 piglets), gender and birthweight (low ≤ 1.1kg; normal ≥ 1.3kg) on the relative absorption of FITC-D (25 mg/kg BW) in the plasma of newborn piglets 2 and 4 hours after ingestion. Data presented as means ± standard error of the mean and defined as statistically significant when P ≤ 0.05.

	Hours post gavage		*P* value
Litter size	2hrs	Smaller 8.49 ± 0.59	*P* = 0.116
		Larger: 9.92 ± 0.64	

	4hrs	Smaller: 14.85 ± 0.85 Larger: 17.37 ± 0.92	*P* = 0.066
Gender	2hrs	Male: 9.52 ± 0.62 Female: 8.89 ± 0.61	*P* = 0.479
	4hrs	Male: 16.52 ± 0.88 Female: 15.52 ± 0.87	*P* = 0.577
Birthweight	2hrs	Low: 9.49 ± 0.60 Normal: 8.92 ± 0.63	*P* = 0.521
	4hrs	Low: 16.83 ± 0.85 Normal: 15.52 ± 0.90	*P* = 0.299

4. Discussion

As glucocorticoids (GCs) are important triggers of intestinal maturation, prenatal exposure to dexamethasone was predicted to influence gut permeability in newborn piglets. It was also assumed that low birthweight piglets would be more likely to suffer from relatively lower gut maturation and be more susceptible to treatment influence. As no significant differences were observed for FITC-D uptake, it is likely that the administration of dexamethasone to gilts on the day of farrowing has no influence on neonatal molecular absorption. Although the dose was on the high end of the therapeutic range (20mg) timing in relation to fetal development is a vital factor for glucocorticoid effects [14,23] and administering dexamethasone to sows on the day of farrowing is likely not the time for any change, be it positive or negative, to occur. A trend towards greater FITC-D absorption and smaller litter size (P = 0.066) was noticed at four hours post gavage. Following the same protocol as Westrom et al. [21] piglets had

three hours opportunity to suckle, although colostrum intake was not measured. With the intake of colostrum being unmeasured, it cannot be certain whether all low birthweight piglets received a similar amount prior to FITC-D ingestion. Jensen et al. [24] found the absorption of macromolecules increased significantly if piglets were given hourly feeds of colostrum (15 mL/kg/h). There is a possibility that piglets born to a smaller litter had a greater opportunity to suckle colostrum prior to FITC-D treatment and a greater capacity for absorption as observed in the study conducted by Jensen et al. [24]

After piglets were gavaged with the FITC-D solution, the concentration of FITC-D in the plasma increased significantly from the second hour to the fourth hour. Jensen et al. [24] and Westrom [21] found absorption of molecular markers increases with time, particularly when piglets ingested colostrum prior to molecular treatment. In our investigation, the absorption of FITC-D was greater than the 1.34% absorption reported by Westrom et al. [21], which may be due to the use of a smaller fluorescently labelled dextran. Although smaller, the dextran used in this investigation (4,000 Dalton) was still large enough to be excluded upon macromolecular closure, as also reported for newborn pigs [21] and adult mice [25]. Further, Woting et al. [25] also reported 70 kDa and 4 kDa molecules moved at same rate through the intestinal wall, although this information was not gathered on our newborn piglets.

The absorption of FITC-D was also no different between normal and low birthweight piglets. Wang et al. [26] found low birthweight piglets had an even greater absorptive capacity for FITC-D (4kDa) but attributed this to compromised barrier function post closure. Although good gut permeability is important for uptake of immunoglobulins, improper gut closure in piglets can lead to bacterial infection and early mortality [26]. As addressed earlier, the severity of growth restriction in utero can be assessed using head morphology [6]. Although all LBW piglets in this study were under 1000 grams at birth, not many were characterized with the distinct 'dolphin' head shape that marks a piglet with severe IUGR. Had more piglets been born with severe IUGR, higher absorptive capacity for FITC-D may have been observed as was seen by Wang et al. [26]. The effect of dexamethasone for growth restricted piglets may not be in the uptake of macromolecules pre-closure, but the ability of the gut to close properly following ingestion of colostrum. Any further investigation into gut performance of

growth restricted piglets should therefore look at gut performance before and after intended closure.

5. Conclusion

The administration of dexamethasone to sows the day of farrowing did not have an effect on the molecular transfer of FITC-D in low birthweight piglets. It is not certain whether synthetic glucocorticoids can improve gut performance in low birthweight piglets, or the timing of treatment needs to be earlier than what was given in this investigation. In future investigations, observing gut performance of low birthweight piglets should not only be a measure of absorptive capacity at birth, but the ability of the gut to close properly after a 24-hour period.

Acknowledgements

We gratefully acknowledge the University of Adelaide and the Australian Pork Ltd for their financial support.

References

1. Miller, Y.; Collins, A.; Smits, R.; Thomson, P.; Holyoake, P. Providing supplemental milk to piglets preweaning improves the growth but not survival of gilt progeny compared with sow progeny. *J. Anim. Sci.* **2012**, *90*, 5078-5085.
2. Smits, R.; Collins, C.F.r.D.-. Measurement and comparison of feed intake and growth performance during lactation and wean-finish of gilt progeny pigs cross-fostered onto multiparous sows. *The Pork Cooperative Research Centre, Australia* **2009**.
3. De Vos, M.; Che, L.; Huygelen, V.; Willemen, S.; Michiels, J.; Van Cruchten, S.; Van Ginneken, C.; nutrition, a. Nutritional interventions to prevent and rear low-birthweight piglets. *J. Anim. Physiol.* **2014**, *98*, 609-619.
4. Craig, J.R.; Hewitt, R.J.; Muller, T.L.; Cottrell, J.J.; Dunshea, F.R.; Pluske, J.R. Reduced growth performance in gilt progeny is not improved by segregation from sow progeny in the grower–finisher phase. *Animal* **2019**, *13*, 2232-2241.
5. Wijesiriwardana, U.A.; Pluske, J.R.; Craig, J.R.; Cottrell, J.J.; Dunshea, F.R. Evaluation of sugarcane-derived polyphenols on the pre-weaning and post-weaning growth of gilt progeny. *Animals* **2020**, *10*, 984.
6. Amdi, C.; Krogh, U.; Flummer, C.; Oksbjerg, N.; Hansen, C.; Theil, P. Intrauterine growth restricted piglets defined by their head shape ingest insufficient amounts of colostrum. *J. Anim. Sci.* **2013**, *91*, 5605-5613.
7. Ekert Kabalin, A.; Balenović, T.; Šperanda, M.; Milinković-Tur, S.; Štoković, I.; Menčik, S.; Maurić, M.; Pavičić, Ž. Serum biochemical parameters in suckling piglets with low and average birth mass. *Veterinarski arhiv* **2017**, *87*, 171-184.
8. Korgun, E.T.; Ozmen, A.; Unek, G.; Mendilcioglu, I. The effects of glucocorticoids on fetal and placental development. *Glucocorticoids-New Recognition of Our Familiar Friend* **2012**.
9. Moisiadis, V.G.; Matthews, S.G. Glucocorticoids and fetal programming part 1: outcomes. *Nat. Rev. Endocrinol.* **2014**, *10*, 391-402.

10. Sangild, P.; Xu, R.; Trahair, J. Maturation of intestinal function: the role of cortisol and birth. In *Biology of Growing Animals*; Elsevier: 2002; Volume 1, pp. 111-144.
11. Barnes, P.J.; Adcock, I.M. Glucocorticoid resistance in inflammatory diseases. *The Lancet* **2009**, *373*, 1905-1917.
12. Drake, A.J.; Walker, B.R.; Seckl, J.R. Intergenerational consequences of fetal programming by in utero exposure to glucocorticoids in rats. *Am. J. Physiol. Regul.* **2005**, *288*, R34-R38.
13. McKinlay, C.J.; Crowther, C.A.; Middleton, P.; Harding, J.E. Repeat antenatal glucocorticoids for women at risk of preterm birth: a Cochrane Systematic Review. *Am. J. Obstet. Gynecol.* **2012**, *206*, 187-194.
14. Seckl, J.R. Glucocorticoids and fetal programming. *Toxicology* **2000**, *148*, 14-15.
15. Schmidt, M.; Sangild, P.; Blum, J.; Andersen, J.; Greve, T. Combined ACTH and glucocorticoid treatment improves survival and organ maturation in premature newborn calves. *Theriogenology* **2004**, *61*, 1729-1744.
16. Le Dividich, J.; Rooke, J.; Herpin, P. Nutritional and immunological importance of colostrum for the new-born pig. *J. Agri. Sci.* **2005**, *143*, 469-485.
17. Oliviero, C.; Junnikkala, S.; Peltoniemi, O. The challenge of large litters on the immune system of the sow and the piglets. *Repro. Domest. Anim.* **2019**, *54*, 12-21.
18. Alonso-Spilsbury, M.; Ramirez-Necoechea, R.; Gonzlez-Lozano, M.; Mota-Rojas, D.; Trujillo-Ortega, M. Piglet survival in early lactation: a review. *J. Anim. Vet. Adv.* **2007**.
19. Bland, I.; Rooke, J.; Bland, V.; Sinclair, A.; Edwards, S. Appearance of immunoglobulin G in the plasma of piglets following intake of colostrum, with or without a delay in sucking. *J. Anim. Sci.* **2003**, *77*, 277-286.
20. Bate, L.A.; Ireland, W.P.; Connell, B.; Grimmelt, B. Development of the small intestine of piglets in response to prenatal elevation of glucocorticoids. *Histol. Histopathol.* **1991**.
21. Weström, B.; Svendsen, J.; Ohlsson, B.; Tagesson, C.; Karlsson, B. Intestinal transmission of macromolecules (BSA and FITC-labelled dextrans) in the neonatal pig. *Neonatology* **1984**, *46*, 20-26.
22. Svendsen, L.; Weström, B.; Svendsen, J.; Olsson, A.-C.; Karlsson, B. Intestinal macromolecular transmission in underprivileged and unaffected newborn pigs: implication for survival of underprivileged pigs. *Res. Vet. Sci.* **1990**, *48*, 184-189.
23. Ramamoorthy, S.; Cidlowski, J.A. Corticosteroids: mechanisms of action in health and disease. *Rheum. Dis. Clin.* **2016**, *42*, 15-31.
24. Jensen, A.R.; Elnif, J.; Burrin, D.G.; Sangild, P.T. Development of intestinal immunoglobulin absorption and enzyme activities in neonatal pigs is diet dependent. *J. Nutr.* **2001**, *131*, 3259-3265.
25. Woting, A.; Blaut, M. Small intestinal permeability and gut-transit time determined with low and high molecular weight fluorescein isothiocyanate-dextrans in C3H mice. *Nutrients* **2018**, *10*, 685.
26. Wang, W.; Degroote, J.; Van Ginneken, C.; Van Poucke, M.; Vergauwen, H.; Dam, T.M.T.; Vanrompay, D.; Peelman, L.J.; De Smet, S.; Michiels, J. Intrauterine growth restriction in neonatal piglets affects small intestinal mucosal permeability and mRNA expression of redox-sensitive genes. *The FASEB Journal* **2016**, *30*, 863-873.

Chapter 5: Development of a novel drug delivery system for sows to control the timing of piglet delivery and improve farrowing supervision.

Statement of Authorship

Title of Paper	Development of a novel vaginal drug delivery system to control time of farrowing and allow supervision of piglet delivery
Publication Status	☒ Published ☐ Accepted for publication ☐ Submitted for publication ☐ Unpublished and Unsubmitted work written in manuscript style
Publication Details	Ward, S. A., Kirkwood, R. N., Plush, K. J., Abdella, S., Song, Y., & Garg, S. (2022). Development of a Novel Vaginal Drug Delivery System to Control Time of Farrowing and Allow Supervision of Piglet Delivery. Pharmaceutics, 14(2), 340. DOI: https://doi.org/10.3390/pharmaceutics14020340

Principal Author

Name of Principal Author (Candidate)	Sophia Ward		
Contribution to the Paper	Conception of project, carried out in vitro experimental procedures (all tablet analyses, swelling and mucoadhesion studies, dissolution and HPLC analysis), ran experimental testing of drug delivery system on sows in vivo, analysed statistics, wrote first manuscript.		
Overall percentage (%)	70 %		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	22/02/2022

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Roy Kirkwood		
Contribution to the Paper	Conception of the project, drafting and evaluation of the manuscript (6%)		
Signature		Date	22/02/22
Name of Co-Author	Kate Plush		
Contribution to the Paper	Assisted with drafting and evaluation of manuscript (4%)		

Signature		Date	22/02/22
Name of Co-Author	Yunmei Song		
Contribution to the Paper	Assisted and supervised in vitro investigations, helped to designed formulations for immediate and extended release, drafting of manuscript (10%)		
Signature		Date	22/02/22
Name of Co-Author	Sadikalmahdi Abdella		
Contribution to the Paper	Assisted with mathematical models for determining release kinetics, helped with proofreading of final manuscript (3%)		
Signature		Date	22/02/22
Name of Co-Author	Sanjay Garg		
Contribution to the Paper	Supervision of in vitro work, assisted in drafting of manuscript (7%)		
Signature		Date	22/02/22

pharmaceutics

MDPI

Article

Development of a Novel Vaginal Drug Delivery System to Control Time of Farrowing and Allow Supervision of Piglet Delivery

Sophia A. Ward [1,] *, Roy N. Kirkwood [1], Kate J. Plush[2]; Sadikalmahdi Abdella[3], Yunmei Song[3] and Sanjay Garg[3]

[1] School of Animal and Veterinary Sciences, University of Adelaide, SA 5371, Australia
[2] Sunpork Group, 1/6 Eagleview Place, Eagle Farm, QLD 4009, Australia
[3] Clinical and Health Sciences, University of South Australia, Australia, Adelaide, SA 5000, Australia
* Correspondence: sophia.ward@adelaide.edu.au

Abstract: The swine industry has evolved significantly in the recent decades, but this has come at considerable expense to piglet survival. Breeding sows for greater prolificacy has been accompanied by a greater proportion of piglets being born underweight, of lower vigor, and higher susceptibility to early mortality. Inducing sows to farrow during working hours has the potential to increase piglet survivability, but non-therapeutic injectable products are often discouraged on farms. We aimed to design and develop a novel vaginal drug delivery system (NVDDS) that could reliably trigger luteolysis and induce parturition. To achieve this, two vaginal tablets containing the luteolytic agent cloprostenol were formulated to be inserted together: one would release constituents immediately on insertion (immediate release; IR) and the other would release cloprostenol in a controlled manner (controlled release; CR). The two formulations (IR and CR) were evaluated for drug release, swelling and bio-adhesion in conditions simulating the sow vaginal environment. The IR tablet released the drug completely for 5 min whereas the CR tablet took 5 h to release 50% of the drug. Furthermore, the release kinetics were evaluated by fitting the dissolution profiles into different mathematical models. Both IR and CR tablets were best fitted by the Makoid–Banakar model which assumes release by summation of different mechanisms. The in vitro performance of the optimized formulations was studied with 161 Large White x Landrace sows of varying parity (0–5). The sows were assigned to five groups. Group 1 (SI) received a single vulval injection of cloprostenol at 0700 h (n = 32), group 2 (SDI) received the same dose split in two parts, at 0700h and 1300h (n = 33). Group 3 (IRT) animals were administered an IR tablet at 0700h (n = 32), while group 4 (IRCRT) received both IR and CR tablets at 0700 h (n = 33). Group 5 was untreated and served as a control (n = 32). The interval to farrowing was longer ($p < 0.001$) for controls than for treated sows, but there were no differences among cloprostenol treatments for timing of farrowing. The finding confirms the efficacy of the NVDDS for induction of farrowing in sows.

Keywords: sow; farrowing; cloprostenol; veterinary; vaginal deposit

1. Introduction

Pre-weaning mortality continues to be an ongoing issue for the pork industry [1-3] with the majority of piglet loss occurring during the first 3 to 7 days of lactation [4-6]. In the absence of human intervention, newborn piglets are susceptible to a multitude of issues during and after farrowing including hypoxia, inadequate colostrum intake, and overlay by sows [7]. Although multiple strategies have been developed to improve early piglet survival [8-11], most are dependent on the presence of farm personnel to supervise the farrowing.

To allow for closer management of the neonatal litter, sows can be induced to farrow to provide a greater likelihood of piglet delivery during working hours. The injection of prostaglandin F2α, or an analogue (e.g., cloprostenol), can trigger regression of the corpora lutea ending progesterone secretion and stimulating piglet delivery within 22–36 h [12]. To increase the likelihood of a terminal luteolysis, treatments can be administered using the so-called split-dose protocol where the product is administered in the morning and again approximately 6 h later [13]. Using the split-dose protocol, the proportion of sows farrowing the following working day increased from 56% to 84% [13]. A graphical explanation of this process is presented in Figure 1.

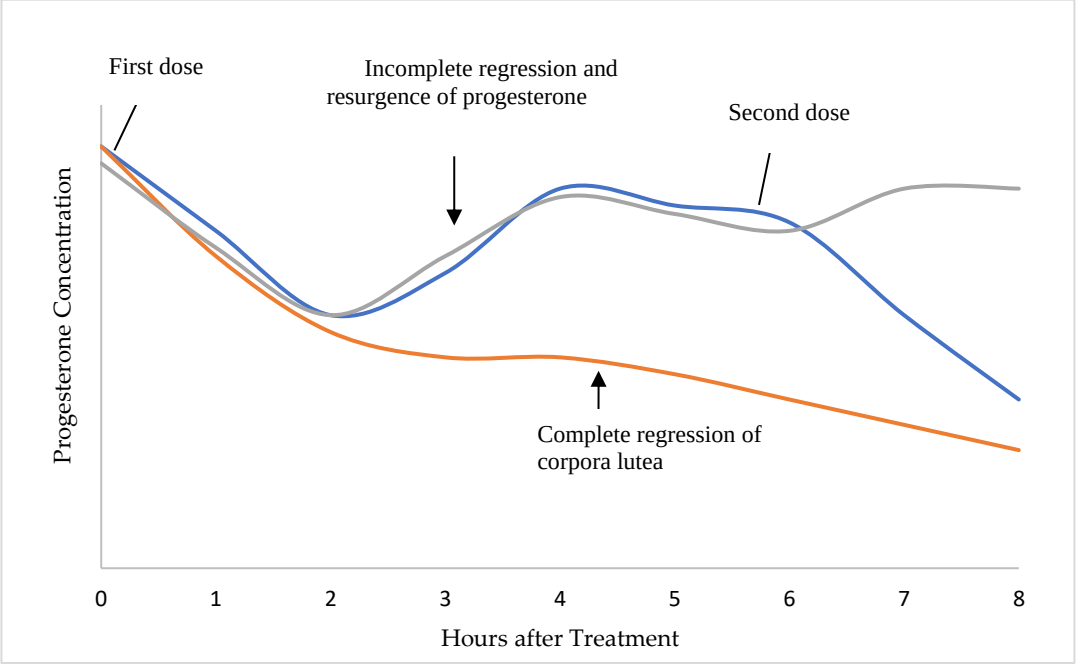


Figure 1. Graphical representation of how progesterone concentrations fluctuate when sows are given a luteolytic agent (i.e., cloprostenol). The first dose can trigger complete regression for successful induction (orange line) or incomplete regression causing an unsuccessful induction (grey line). Administering a second luteolytic dose 6 hours after the first should trigger complete regression and result in a successful induction (blue line)[12-14]

Provided the treatment is given within the final two days of the herd-specific due date, induction of parturition is a safe and effective measure for improving piglet survival

[7,8,15]. However, in commercial practice, a major constraint in the use of induction is the need for injectable treatments that can be acutely painful and stressful to sows [15]. For this reason, many commercial farms are against the use of injectables for non-medicinal purposes, which limits the potential for luteolytic agents in production.

The present study describes the development of novel vaginal drug delivery systems (NVDDS) containing the synthetic prostaglandin, cloprostenol. The NVDDS was designed to control the release of cloprostenol after tablet placement in the sow vagina. Super-disintegrants are often included in IR tablets to achieve faster disintegration [16]. For the controlled release (CR), the drug release rate from the tablet can be controlled over time using slow-release polymers either alone or in combination. A hypothetical result for IR and CR NVDDS is presented in Figure 2.

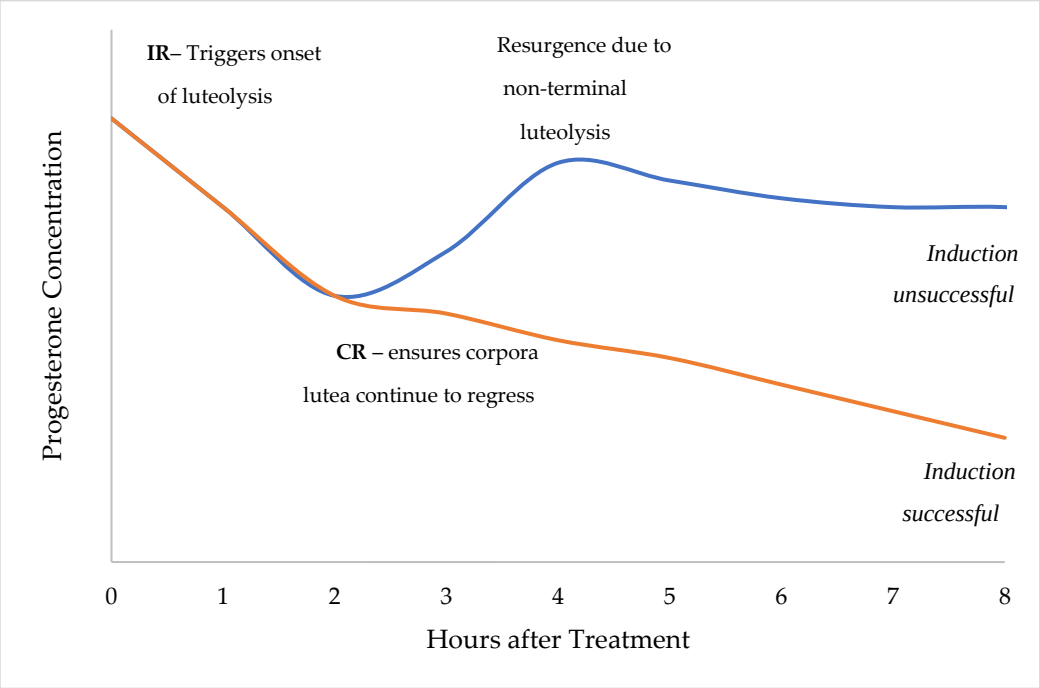


Figure 2. Graphical representation of how progesterone concentrations in sows are predicted to respond to the IR and CR formulations. An ideal RR NVDDS would trigger onset of luteolysis (orange and blue line). Administering the CR NVDDS should release cloprostenol gradually, triggering complete regression and result in a successful induction (orange line). Without the CR NVDDS, risk of resurgence due to non-terminal luteolysis could result in an unsuccessful induction (blue line).

To achieve the desired release profile of cloprostenol from CR, hydroxypropyl methylcellulose (HPMC) can be used for its biodegradability and non-toxic properties [17,18]. This cellulose-based polymer will swell and form a gel-like matrix to control release of an incorporated drug into the surrounding fluid. Different viscosity grades and quantities of HPMC will determine how well the polymer will relax with volume expansion, allowing cloprostenol, a highly water-soluble drug, to diffuse out of the tablet matrix [19,20]. The CR NVDDS must not only release optimally but should be

mucoadhesive while the drug is releasing to ensure retention. Different concentrations and gradients of HPMC and lactose should be compared to identify an optimal formulation. Previous reports determined that a 50% dose [13,21] of prostaglandin or its analogue injected into the vulva was as effective as an intramuscular injection at the full label dosage. Therefore, in the present study, NVDDS was formulated to deliver relatively low cloprostenol into the vagina either immediately after insertion (IR) or to release in a controlled manner over a sustained period (CR). Our hypothesis was that the double administration of cloprostenol via IR and CR NVDDS will be just as effective at inducing sow parturition as the traditional 'split dose' [12,14] intravulval delivery protocol.

2. Materials and Methods

2.1. High Performance Liquid Chromatography (HPLC)

Using the method outlined by Kalikova et al. [22], enantiomers were separated and quantified. The separation system consisted of a Lux Cellulose-1 column (Phenomenex, Pty, Ltd, Lane Cove, NSW Australia) acetonitrile-sodium dihydrogenphosphate (pH 3; 20 mM) (1:2, v/v) as the mobile phase. HPLC was performed using a Shimadzu LC system (Shimazu Corporation, Kyoto, Japan) with a column temperature set to 20 °C, a flow rate of 0.7 mL/min, and a wavelength 274 nm. Retention time of cloprostenol was 6.5 min (linearity range: 2.5–30 µg/mL; r2 = 0.99) as presented in Figure 3.

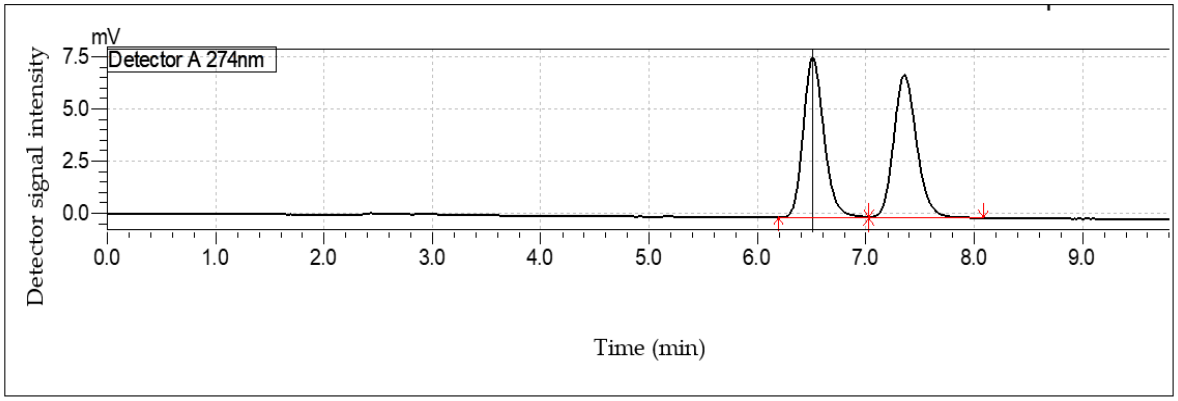


Figure 3. HPLC chromatogram of (±)-cloprostenol was separated into its two enantiomers using the method outlined by Kalikova et al. [22]

2.2. NVDDS Preparation

The IR tablet was formulated to release constituents immediately on contact with biological fluid and be sturdy enough to resist tablet breakage prior to use. Kiccolate ND-2HS (5.4 mg) was used as a super disintegrant, Magnesium stearate (1.8 mg) and Aerosil 200 (1.8 mg) were added for improving flowability, and Microcrystalline cellulose KG-802 (19 mg) was used for tablet compressibility. Mannitol (60 mg) and anhydrous lactose (85.5 mg)

were used as fillers to obtain the desired final weight of each tablet (186 mg). These compounds were shaken thoroughly before a racemic mixture of cloprostenol (125 μg) was ground into the mixture. To ensure even drug dispersion, tablet mix was incorporated with cloprostenol in small amounts and shaken thoroughly between each addition. For the CR tablet, cloprostenol (125 μg) was combined with Magnesium stearate (1.8 mg), Aerosil 200 (1.8 mg), Microcrystalline cellulose PH-102 (19 mg) and Mannitol (60 mg). Between CR formulations, different viscosity grades of HPMC and ratios of HPMC and anhydrous lactose were tested, as presented in Table 1.

Table 1. Composition of each tablet in controlled-release formulations (CR1–CR6). Hypermellose was obtained from the Dow Chemical Company as METHOCELL K100 Premium CR, K15M Premium CR, K4M Premium CR, and E50 Premium.

Formulation	Lactose (mg)	HPMC K100 (mg)	HPMC K15 (mg)	HPMC K4 (mg)	HPMC E50 (mg)	HPMC % (*w*/*w*)	Lactose % (*w*/*w*)
CR1	85	20	-	-	-	10	46
CR2	85	15	5	-	-	10	46
CR3	60	-	15	30	-	24	32
CR4	55	-	-	50		27	29
CR5	60	-	20	-	20	23	32
CR6	55	-	-	30	20	26	29

Once all the tablet ingredients were thoroughly mixed in a polythene bag, the tablets were prepared by direct compression method.

For each formulation, ten tablets were weighed, measured for thickness using a vernier caliper (Copley Scientific, Colwick, Nottingham, UK) and hardness tested using a digital force gauge (Electrolab model EH-01P; Cupertino, CA, USA) for peak breaking point (KPI).

2.3. *Friability Test*

For the selected formulations IR and CR6, a sample of tablets (n = 35) was taken from the batch, dedusted, and weighed together. Tablets were then placed in a dual drum tablet friability tester (EF-2 Friabilator USP, Electrolab Pty, Ltd., Goregaon East, Mumbai, India) rotated 100 times and dedusted prior to reweighing. The difference in weight before and after was expressed as the percentage lost.

2.4. *Drug Content Uniformity*

For the selected formulations IR and CR6, a sample of tablets (n = 10) was taken from the batch. Each tablet was dissolved in a 100 mL volumetric flask of Milli-Q water and assessed for total drug content using HPLC. The acceptance value was calculated using the formula:

$$(M - X) + k \times s$$

where M = reference value, X = mean of individual contents, k = acceptability constant (2.4), and s = standard deviation.

2.5. Dissolution Study

To assess the release behaviour of the developed formulations, each tablet was placed into a vial containing 10 mL of Milli-Q water and the vials were rotated at 25 rpm at 37 ± 1 °C [23]. One millilitre of samples was withdrawn at each sample time. The medium was kept at a constant volume by refilling it with fresh water. The withdrawn samples subsequently were filtered through a 0.45 μm filter and assayed by HPLC.

Release studies were conducted on each formulation with three tablets and average values were plotted against time.

2.6. Polymer Swelling for CR NVDDS

To assess tablet bio-effectiveness in vitro, the artificial vaginal solution was formulated to simulate the pH and temperature of sow vaginal secretions in late gestation (Appendix B). Simulated porcine vaginal fluid (PSVF) was prepared using the composition reported by Owen and Katz for humans [24] and adjusted with NaOH to a pH of 7. The pH was adjusted to suit the vaginal conditions of Large White x Landrace sows in late gestation (Appendix B) as well as reports on porcine vaginal environment by Lorenzen et al. [25].

The rate of medium uptake for each tablet formulation was assessed using the method of Chaibva et al. [26]. Dry tablets were weighed using an electronic balance and fixed onto a pre-weighed plastic square. The tablets were covered with 10 mL of PSVF and rotated at 10 rpm at 37 ± 5°C for ten hours. Every hour, tablets were removed from PSVF, blotted lightly with kimtech tissue paper, and weighed. Swelling was assessed using the following equation:

$$\text{Swelling (\%)} = \{(W_t - W_o)/ W o\} \times 100$$

where Wo = dry tablet weight and Wt = swollen tablet weight.

2.7. CR NVDDS Bio-Adhesion Test

Porcine vaginal tissue was obtained from a local slaughterhouse (Murray Bridge, SA, Australia) and transported in PSVF (pH = 7) on ice to the laboratory. The vaginal tissue was removed from surrounding tissue, rinsed three times with isotonic saline solution [27], and stored in aluminium foil at −20 °C for later analysis as described by Hiorth et al. [28].

CR tablets were fixed onto a stainless-steel probe with cyanoacrylate adhesive on a hydraulic press. Porcine mucosa was thawed in PSVF (pH 7) at 37 ± 1 °C for 60 min using a magnetic stirrer and heated disk [28] and secured into the mucoadhesion rig suspended in PSVF at 37 °C ± 1 °C. The TA.XTplus texture analyser (Arrow Scientific, Pty, Ltd, Gladesville, NSW, Australia) evaluated bio-adhesion force, with a schematic diagram presented in Figure 4.

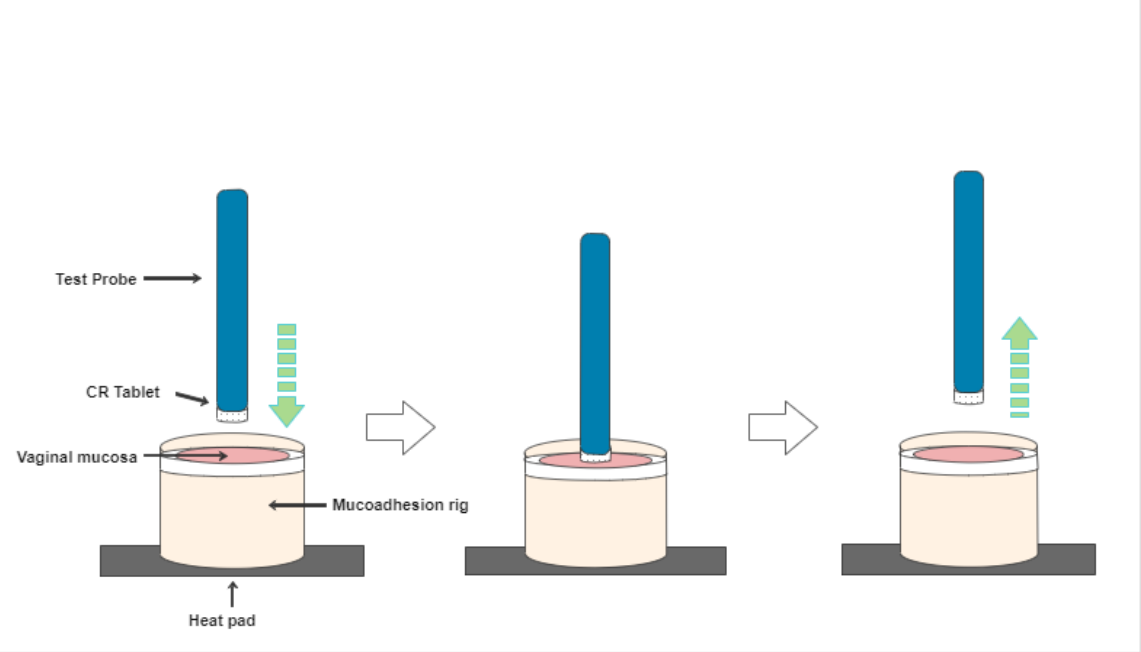


Figure 4. TA. XT PLUS texture analyzer set up. The vaginal mucosa was cut to appropriate size to fit mucoadhesion rig support ring and the probe. Prior to testing, the vaginal mucosa was hydrated in PSVF for 15 min at 37 ± 1 °C.

Following the method of Hiorth et al. [28] for the evaluation of vaginal tablets, the probe with the attached tablet was moved down to the tissue at 1 mm/s until contacting the vaginal mucosa, applying a contact force of 5.0 g for 30 s. The probe was then separated at a speed of 0.1 mm/s until the tablet was detached (*Fmax*). Each batch was evaluated in triplicate.

2.8. In vivo testing of NVDDS

After assessing the activity of selected formulations, the chosen CR (CR6) and the IR tablets were tested in vivo using sows housed at the University of Adelaide Roseworthy Piggery with approval from institutional ethics committee (AECS09:34706). Approximately seven days before farrowing, Large White x Landrace sows (n=161) were moved into individual farrowing crates where they remained for the duration of the trial. Sows had free access to

fresh water and were fed twice daily with a standard pelleted diet formulated to meet all nutrient requirements. Two days before their expected due date (day 113 of gestation), sows were assigned to one of five treatments: SI, SDI, IRT, IRCRT or control.

- **SI:** Injection of 250 μg cloprostenol (Juramate®, Jurox Pty, Ltd, Rutherford, NSW, Australia) into the vulva at 0700 hours;
- **SDI:** Injection of 125 μg cloprostenol into the vulva at 0700 hours and again at 1300 hours;
- **IRT:** Insertion of IR tablet at 0700 hours and again at 1300 hours; For each vaginal deposition, the tablet applicator was sanitised (F10SC Veterinary Disinfectant), rinsed with water and lubricated (Obstetrical Lubricant, ZebraVet, Sherwood, QLD, Australia)
- **IRCRT:** Insertion of IR and CR (formulation CR6) tablets at 0700 hours.
- **Control:** No cloprostenol administration

Data recorded were the interval from treatment administration to the delivery of the first piglet (min), total born litter size, piglet birthweights and piglet pre-weaning mortality.

2.9. Statistics

A one-way ANOVA was used to analyse in vitro data using IBM SPSS v20 statistical software package and data are presented as mean ± standard error of the mean. Confidence limit was set at 95% ($P < 0.05$). In vivo sow data were assessed using a general linear mixed model with the random terms room (Identical farrowing rooms 1 - 5) Sow ID and farrowing batch (April, May or June 2021) and fixed effects parity group (P0 - P5) and treatment.

3. Results

3.1. Tablet physical properties

IR and CR tablet thickness and hardness results are presented in Table 2.

Table 2. Physical properties of tablets for the immediate release and controlled-release formulations (CR1–CR6) (n = 10).

Formulation	Thickness (cm)	Hardness (kgf)
IR	2.54 ± 0.00	4.39 ± 0.12
CR1	2.44 ± 0.00	12.96± 0.47
CR2	2.47 ± 0.00	13.25 ± 0.4
CR3	2.53 ± 0.01	13.96 ± 0.15
CR4	2.56 ± 0.00	13.67 ± 0.12
CR5	2.55 ± 0.01	13.24 ± 0.12
CR6	2.55 ± 0.00	13.74 ± 0.13

3.2. Dissolution studies

3.2.1. IR-NVDDS

The IR-NVDDS disintegrated within 5 min of contact with surrounding fluid, with complete release of cloprostenol (Figure 5). Incorporating the super-disintegrant, Kiccolate, provided instantaneous disintegration [30] to enhance drug dissolution rate.

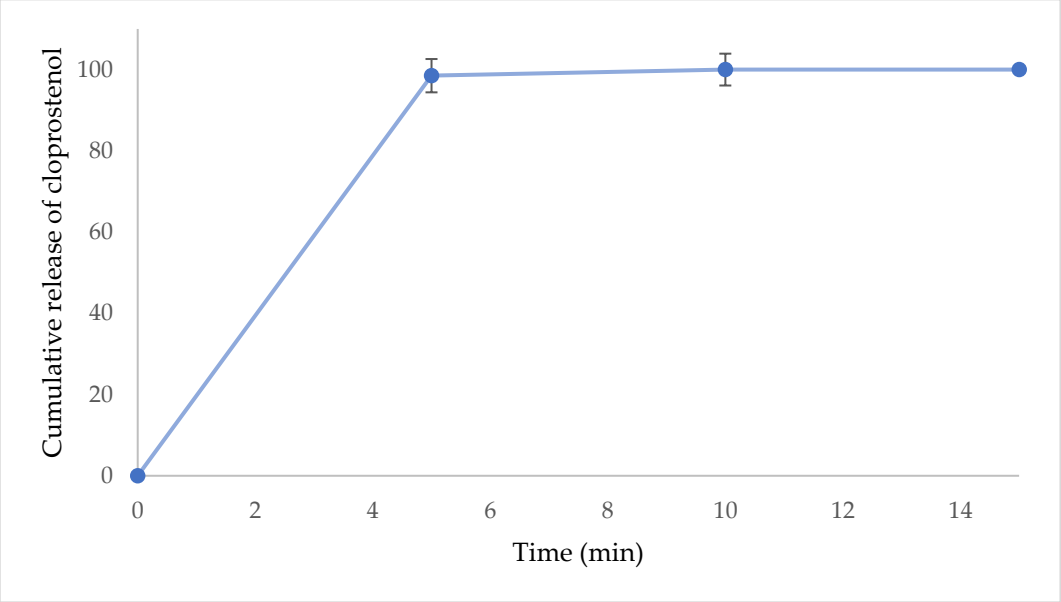


Figure 5. In vitro release profile of cloprostenol IR formulation. Each data point is mean ± standard error of the triplicate.

3.2.2. CR-NVDDS

The CR tablet formulations released cloprostenol gradually over a six or eight-hour period, with dissolution profiles presented for the formulations CR1 – CR6 (Figure 6). Formulations CR1 and CR2 had the highest viscosity grade (K100) of all formulations but the lowest proportion of HPMC in each tablet. Combining lower viscosity grade (K15 and K4) with double the amount of HPMC (CR3) resulted in a slower release after the fourth hour. For CR5 and CR6, incorporating E50 with K15 and K4, respectively, produced optimal release of cloprostenol at the desired rate with ~50% release by the fifth hour. CR6 achieved the target release profile and was selected for the in vivo study.

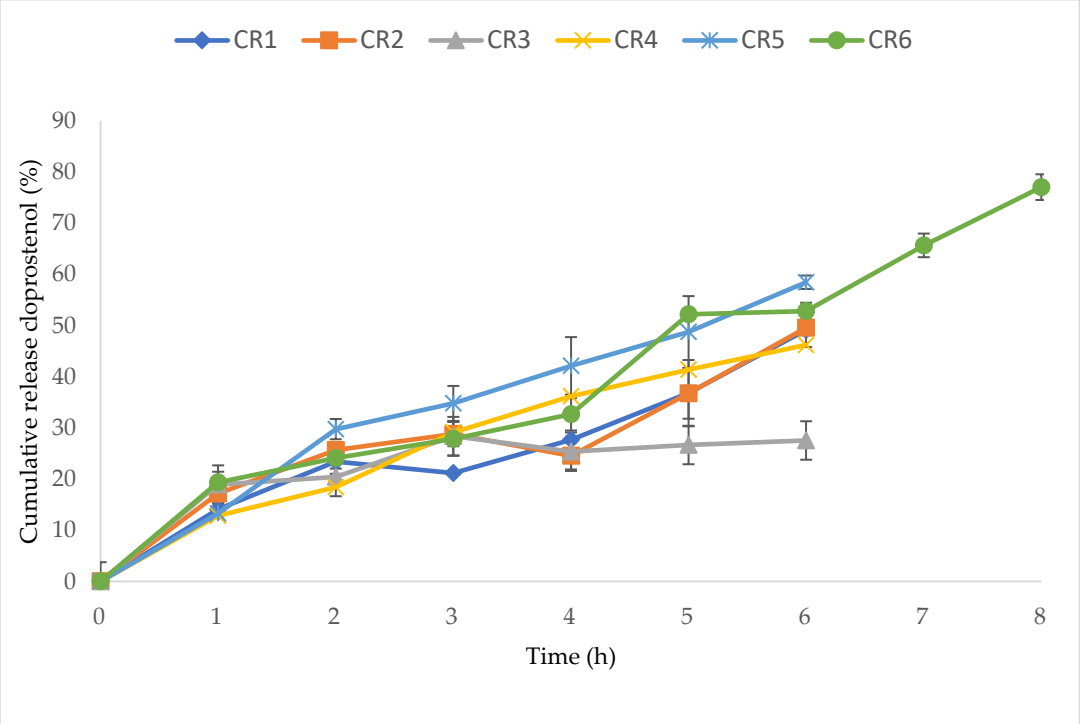


Figure 6. In vitro release profiles of cloprostenol CR NVDDS formulations. Each data point is the mean ± standard error of the triplicate. Formulation CR6 displayed favorable release and was assessed over an eight-hour time period.

3.3. Mechanism of drug release

Mathematical models are employed to better understand the mechanism of drug release from dosage forms. The mathematical model that best fits the dissolution data helps to predict the release kinetics of the drug. To understand mechanism of cloprostenol release from the IR and CR6 tablet formulations, several mathematical models were employed to fit the experimental data to the theoretical curve (see Appendix A). The data from the dissolution studies conducted were fitted into the individual kinetic models and the goodness of fit of the experimental release with predicted release profile was evaluated using three common statistical criteria in combination; the adjusted R2, the RMSE, and the AIC.

The release profile of cloprostenol from IR tablets was fitted by first order, Hopfenberg, Peppas-Sahlin and Makoid-Banakar release kinetics, with R2 value of 1.00. First order release kinetics describe the drug release rate from the pharmaceutical dosage form is proportional to the amount of drug remaining in its interior. The amount of drug released decreases by unit of time. The Makoid-Banakar release model, on the other hand, assumes total drug release is the result of several mechanisms such as burst release, controlled and diffusional release among others. Hopfenberg assumes that the rate-limiting step of drug release is the erosion of the matrix itself while, in Peppas-Sahlin, the drug release is controlled by both Fickian diffusion and case II relaxations. Comparing the models using other statistical criteria, Makoid-Banakar perfectly fits the release of the drug from IR tablets. The release of the drug from IR tablet, in our case, could be due to a burst release. Release of the drug from CR6 tablet was best described by a Makoid-Banakar release model with an adjusted R2 value of 0.9776 (Figure 7).

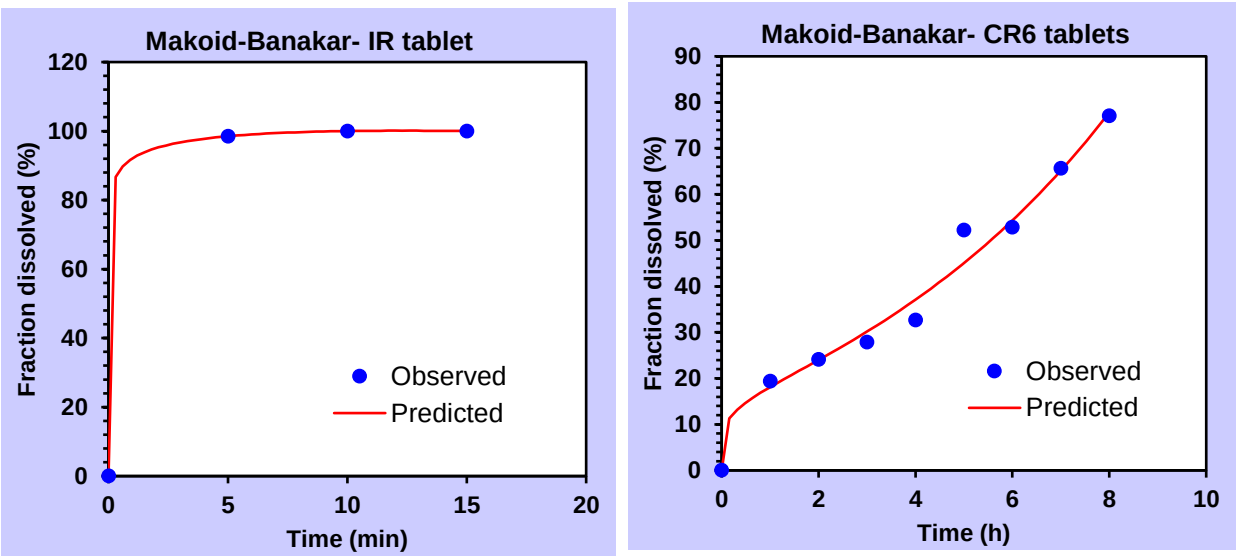


Figure 7. A release profiles of cloprostenol tablets fitted by Makoid-Banakar model.

3.4. Swelling tests

The swelling profiles for NVDDS formulations CR1-CR6 are shown in Figure 8. Formulation CR1 exhibited non-uniformity between tablets, indicated by large error bars. After the eighth hour, the tablet weights decreased as the matrix erodes. Formulations CR5 and CR6 show similar swelling profiles that gradually increases from hours one to eight and then decline in the hours following as the formulation starts to erode.

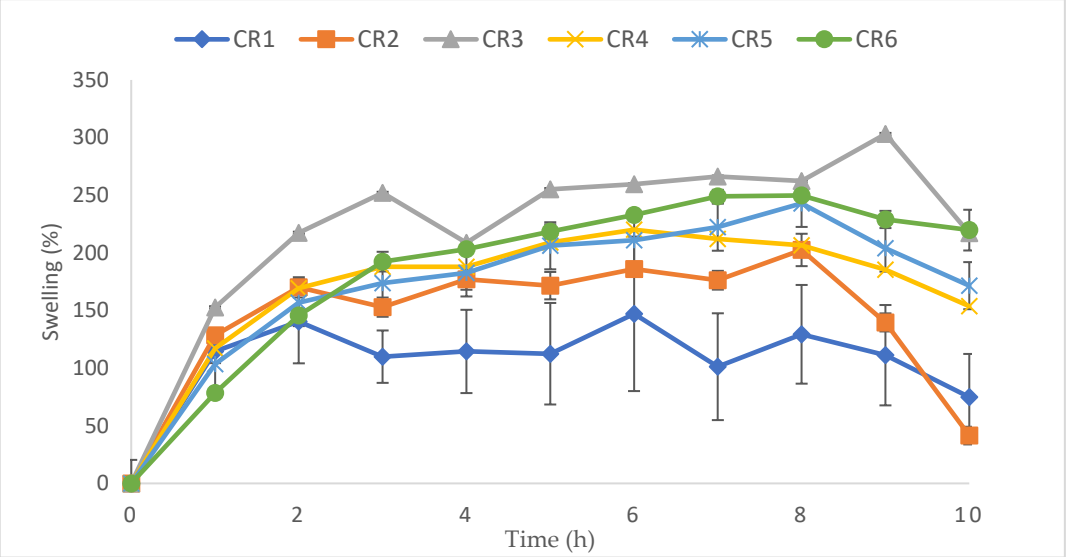


Figure 8. Swelling profiles for controlled release formulations CR1–CR6. Each data point is mean ± standard error of the triplicate.

The visual differences between formulations containing a low concentration of HPMC (CR1), and the desired formulation containing 26% HPMC (CR6), are presented in Figure 9. CR6 exhibited even swelling within the triplicate across hours 1, 5 and 10 (Figure 9a). For CR1, physical differences were observed between the tablet triplicates with uneven tablet swelling and erosion (Figure 9b).

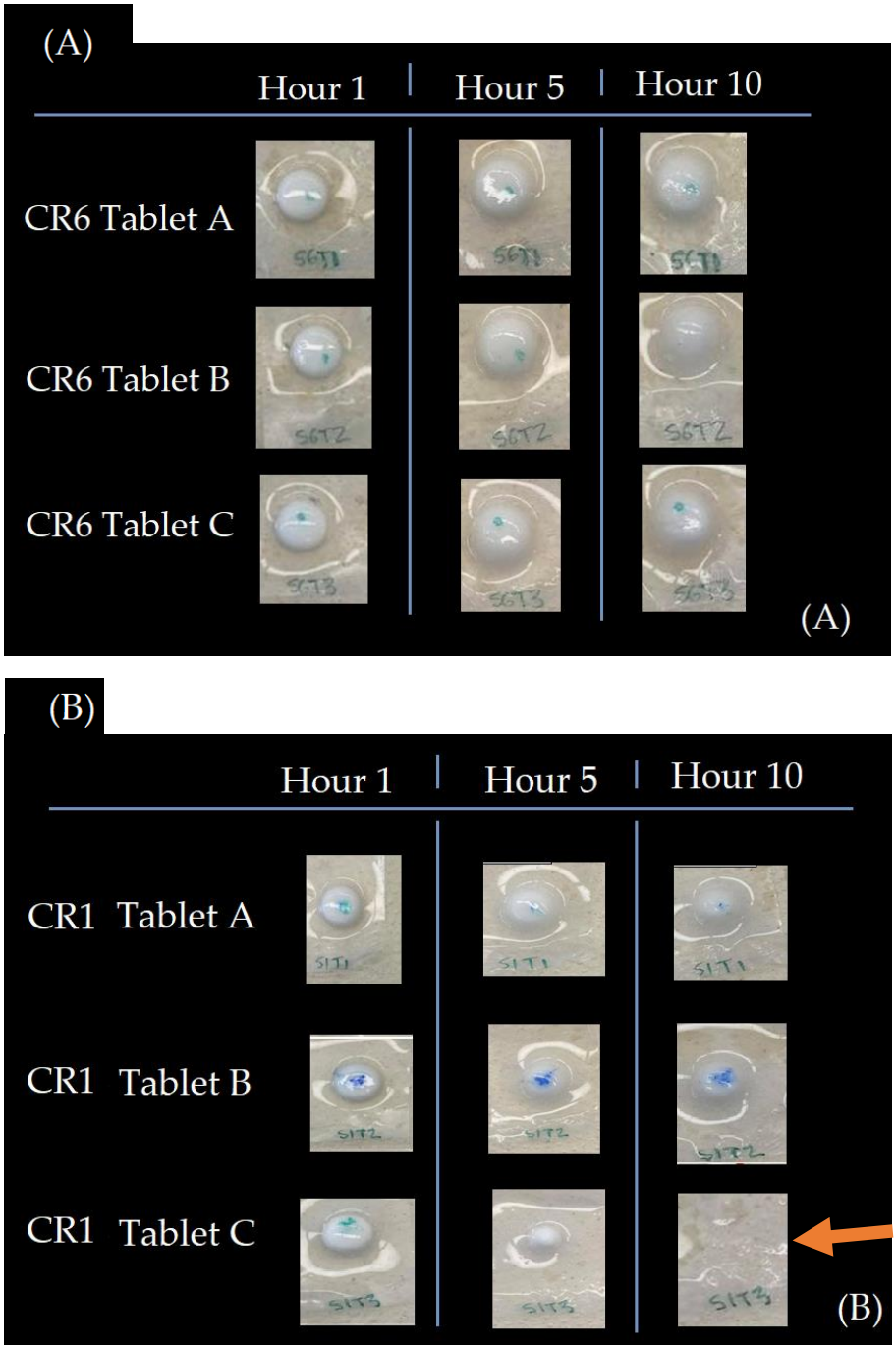


Figure 9. Formulation CR6 (A) and CR1 (B) after 1, 5 and 10 hours of being submerged in PSVF. Uneven tablet erosion is evident between CR1 triplicates (B), as indicated by the arrow.

3.5. Bio-adhesion tests

Following the method outlined by Hiorth et al [29] for vaginal drug delivery in women, we determined the maximum detachment force for testing CR formulations. The values for CR2- CR6 (Figure. 10) were similar to those reported in the study for HPMC based tablets (0.14 ± 0.9 Fmax [N]). CR3 and CR6 had the highest mean detachment forces but were still within the values reported by Hiorth et al. [29]. Formulation CR1 had a

significantly lower detachment force than other formulations (0.06 ± 0.01 Fmax [N]) and a lower value than what was reported by Hiorth et al. [29].

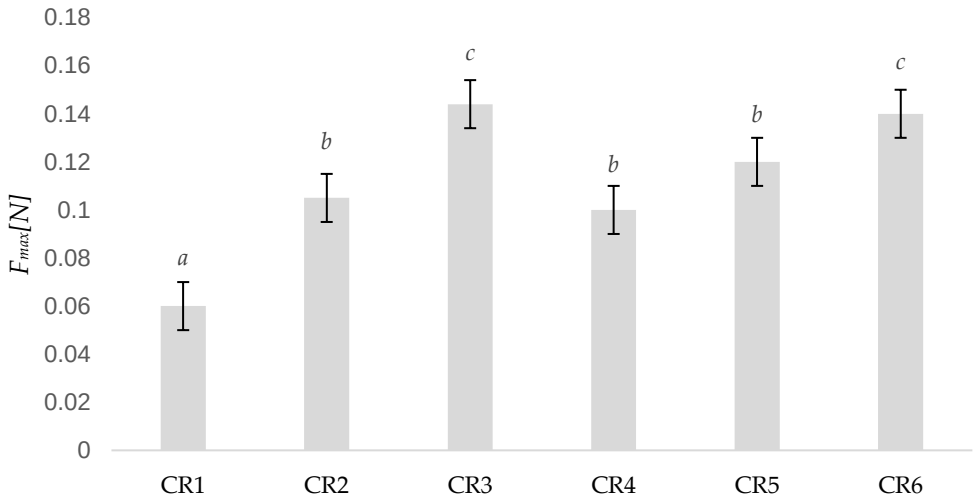


Figure 10. Mean detachment force Fmax (Newtons) for controlled release formulations CR1- CR6 as measured by a TA.XT plus Texture Analyzer (n=3). Formulations with significant differences in mean detachment force (at the 95% level of confidence; P value < 0.05) are represented by different superscripts (*a, b* or *c*).

3.6. Selected formulations uniformity and friability tests

From the results of previous in vitro testing, NVDDS formulation CR6 was selected to test in sows along with the IR NVDDS. The formulated tablet batches selected for in vivo study were assessed for tablet uniformity with test results presented in Table 3. The content uniformity scores for selected tablets (n=10) fit within the maximum allowed acceptance value (L1 ≤ 15 for solid dosage tablets).

Table 3. Testing selected formulations for tablet uniformity (n=10).

NVDDS Formulation	Desired drug Content (μg)	Average content (μg)	Standard Deviation	Uniformity value score (L1 ≤ 15)
Immediate Release (IR)	125	127.4	4.87	10.45
Controlled Release Formulation 6 (CR6)	125	126.7	6.52	13.92

Friability testing was also conducted on selected formulations with no physical signs of cracking, cleaved or broken tablets after 100 rotations in tablet friabilator. The percentage of tablet loss (n=35 tablets) must fit within the maximum acceptance value (Percentage lost % ≤ 1%). Values are presented in Table 4**.**

Table 4. Testing selected formulations for tablet friability (n=35).

NVDDS Formulation.	Original weight (g)	Weight after 100 rotations (g)	Percentage lost (%) ≤ 1%
Immediate Release (IR)	6.591	6.586	0.075
Controlled Release Formulation (CR6)	6.503	6.495	0.123

3.7. In vivo tests

There were no differences among treatments for sow parity, mean litter size, average piglet birthweight or preweaning survival rate, as presented in Table 5.

Table 5. Effects on sow performance of cloprostenol administered either as a single vulval injection at 0700 h, vulval injection at both 0700 and 1300 h, insertion of a rapid release (IR) NVDDS at 0700 and 1300 h, or both IR and controlled release (CR6) tablets at 0700 h or untreated controls. The statistical significance of mean values are presented as a P-value, where P ≤ 0.05 indicates a significant result at the 95% level of confidence.

	Control	SI	SDI	IRT	IRCRT	P–value
Parity	2.63 ± 1.6	2.56 ± 1.6	2.25 ± 1.6	2.30 ± 1.6	2.03 ± 1.6	0.585
Total Born	11.94 ± 0.5	12.47 ± 0.6	13.38 ± 0.7	12.48 ± 0.3	12.6 ± 0.2	0.486
Born Alive	11.25 ± 0.4	11.66 ± 0.5	12.28 ± 0.6	11.73 ± 0.3	11.94 ± 0.4	0.730
Birthweight (kg)	1.38 ± 0.05	1.44 ± 0.04	1.28 ± 0.05	1.36 ± 0.05	1.30 ± 0.02	0.272
Preweaning survival (%)	90.9 ± 1.99	91.43± 1.58	91.61± 1.51	90.04± 2.67	89.89 ± 2.05	0.962

All sows receiving cloprostenol, regardless of dose or route of administration, had shorter intervals to farrowing than the control sows (P = 0.001). The average times to farrow after treatment administration were all within the 23 – 32 hours expected for

successful farrowing induction [14]. No significant differences were observed between sows that were induced by injection or IM and CR tablets (Figure 11). Sows that received a single injection of cloprostenol had the same induction success rate as those that received a split dose.

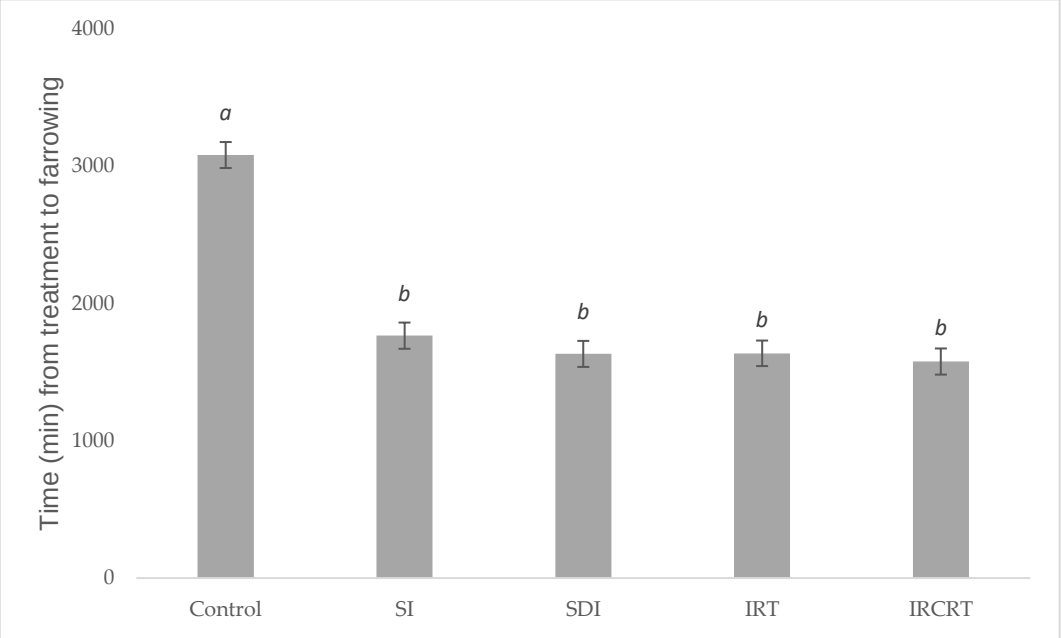


Figure 11. Time taken (minutes) for sows to farrow from time of treatment application to birth of the first piglet. The sows were assigned to five groups. Group 1 (SI) received a single vulval injection of cloprostenol at 0700 h (n=32), group 2 (SDI) received the same dose split in two parts, at 0700 and 1300 h (n=33). Group 3 (IRT) animals were administered an IR tablet at 0700 h (n=32), while group 4 (IRCRT) received both IR and CR tablets at 0700 h (n=33). Group 5 was untreated and served as a control (n=32). Formulations with significant differences in mean detachment force (at the 95% level of confidence; P value < 0.05) are represented by different superscripts (a, b or c).

4. Discussion

The dissolution test results of IR tablet demonstrated the formulation achieved the rapid release of cloprostenol. The super-disintegrant (Kiccolate) resulted in rapid and complete breakdown of the tablet when hydrated by aqueous media (PSVF). Adding microcrystalline cellulose (PH102) into the IR formulation strengthened the tablet at lower compaction forces [31] and provided a sturdy tablet that could still break down rapidly in biological fluid. The developed formulations also resisted chipping or capping after 100 spins in the Tablet Friabilator; not obviously cracked, cleaved, or broken tablets were present in the tablet testing samples after tumbling.

The drug release profiles of CR tablet formulations showed both the viscosity grade and quantity of HPMC polymer contributed to tablet release properties. CR3 had lower HPMC viscosity grade than CR1 and CR2, but a higher concentration of HPMC, resulting in slower release after the fourth hour. Similar results have been reported in other studies [32,33] with higher concentrations and viscosity grade slowing down the release of water-soluble actives. Increasing the concentration of HPMC allows more molecules to pack together and form a matrix with greater viscosity [33]. Reducing the HPMC viscosity grade and keeping the higher concentrations of HPMC provided an even release of cloprostenol into the dissolution fluid every hour. Similarly, Vueba et al. [34] found the combination of high and low HPMC viscosity grades increased gel heterogeneity and provided stable uptake of fluid. Finer HPMC particles allow rapid and uniform hydration of HPMC molecules and a more controlled drug release over time [35].

With the lowest concentration and highest viscosity grade of HPMC, formulation CR1 presented an uneven release of cloprostenol and an unreliable swelling profile between tablet samples. The concentration of HPMC in this formulation (10%) was lower than the 'percolation threshold' reported in other studies [32,36] and may have contributed to the difference in swelling percentage observed in Figure 9(B). Although HPMC concentrations as low as 10% (w/v) have been reported [19,37-39], low levels of HPMC and high lactose content can be associated with sudden changes in matrix integrity and inconsistent results [39,40]. Substances with higher particle size require higher concentrations to percolate the tablet [40,41], so combining with lower viscosity grade may improve tablet robustness. In Figure 8, formulations CR1 and CR2 showed signs of erosion after the eighth hour yet CR3 retained a strong gel matrix with no signs of core erosion. At the surface of the gel tablet layer, formulations containing greater concentrations of HPMC are less susceptible to erosion as there are more polymetric chains to withstand forces surrounding the gel [38]. Concentrations of HPMC below the critical polymer concentration cannot withstand the shear forces surrounding the gel for as long, and erosion occurs at a faster rate [42].

In addition to having an unstable release, CR1 also had the weakest detachment force. CR6 and CR3 had higher HPMC concentrations (>24%) and the higher concentration have also been found to increase mucoadhesive strength in previous studies [38,43]. Increasing the concentration of HPMC in tablet formulations increase the likelihood of

chain entanglement between the hydroxl groups and amide groups of the mucin layer [39].

The optimized formulation for controlled release of cloprostenol was CR6, containing both high and low viscosity grades of HPMC (E and K) and a higher concentration of HPMC (26%, w/v). The results of in vitro testing indicate a combination of viscosity grades and a concentration within reported percolation thresholds for HPMC is important for a reliable, even release rate of cloprostenol.

In vivo studies with periparturient sows indicated a successful release of cloprostenol from tablets and diffusion through vaginal mucosa to initiate luteolysis. No differences were observed between single and double injection of cloprostenol, indicating the treated sow herd did not experience incomplete luteolysis.

The insertion of the IR NVDDS was able to successfully induce farrowing 24-34 hours after application. At the very least, the IR tablet was able to disintegrate and release cloprostenol, and enough of this cloprostenol permeated through the vaginal mucosa for luteolysis to be triggered. Whether the CR6 tablet helped to prevent incomplete luteolysis during this period requires further testing on larger sow herds.

The induction of parturition had no effect on birthweight, piglets born alive or percentage of preweaning survival. Results are similar to those previously reported for survival of the litter for sows induced two days before intended due date [44]. Straw et al. [39] found lighter birthweights for induced sows but similar weights by day 12. Conversely, Gunvaldsen et al. [38] reported similar birthweights between induced and non-induced litters, but found average daily gain was lower for piglets born to induced sows [45]. Further investigation into growth rate of piglets vs number of days in gestation is recommended to understand the possible side effects of implementing an induction protocol in commercial production.

5. Conclusions

Vaginal formulations offer potential for a non-injectable route for cloprostenol to successfully induce sows to farrow during the working day. The immediate release NVDDS formulation was able to disintegrate and release 100% of active constituents within 5 min and tablets did not show obvious signs of being cracked, cleaved, or broken in friability tests. Formulating a sustained release NVDDS with 26% HPMC (w/v) and a

combination of polymers K15 and E50 produced a tablet with a desirable, even release of cloprostenol, resistant to early erosion, tablet uniformity and appropriate bio-adhesion in vitro. As the sample of sows used in this study did not show signs of incomplete luteolysis, it is not evident how effective IR and CR tablets are for reliably inducing farrowing over a single IR dose. Future investigations should test the effectiveness of IR and CR NVDDS on larger sow populations, particularly where there is evidence of single dose administrations of cloprostenol being not as reliable for inducing sows over the split dose method.

Author Contributions: Conceptualization, R. K and S. G; methodology, Y.S; investigation, S.W, writing-original draft preparation, S.W; writing-review and editing, R.K, K.P, S.G, H.A and Y.S; supervision, R.K, K.P, S.G and Y.S. All authors have read and agreed to the published version of this manuscript.

Funding: This research was funded by the University of Adelaide and Australian Pork Limited.

Institutional Review Board Statement: The University of Adelaide Animal Ethics Committee approved this study (34706: AECS09).

Informed Consent Statement: Not Applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Acknowledgments: The authors would like to thank the Pharmaceutical Innovation and Development Group (PIDG) at the University of South Australia for their outstanding support, teaching, and guidance in the development of the NVDDS. We also gratefully acknowledge the University of Adelaide and Australian Pork Limited for their financial support.

Conflicts of Interest: The authors declare no conflict of interest.

Sample Availability: Samples of the compounds not available from the authors.

Appendix A: Release parameters of fitted experimental data for cloprostenol IR and CR 6 tablets.

Model name	Equation	Goodness of fit parameter	IR	CR 6
Zero order	F = k0 × t	R2 adjusted	0.4457	0.9599
		RMSE	1372.1250	24.1740
		AIC	35.2909	49.3825
First order	F = 100 × [1 - Exp(-k1 × t)]	R2 adjusted	1.0000	0.9359
		RMSE	0.0002	38.5851
		AIC	−28.3566	53.5908
Higuchi	F = kH × t0.5	R2 adjusted	0.8308	0.8815
		RMSE	418.8915	71.3687
		AIC	30.5449	59.1257
Hixson-Crowell	F = 100 × [1 - (1 - kHC × t)3]	R2 adjusted	0.9695	0.9494
		RMSE	75.5287	30.4404
		AIC	23.6925	51.4569
Hopfenberg	F = 100 × [1 - (1 - kHB × t)n]	R2 adjusted	1.0000	0.9548
		RMSE	0.0000	27.1928
		AIC	−245.5330	51.2398
Makoid Banakar	F=kMB × tn × Exp(-k × t)	R2 adjusted	1.0000	0.9776
		RMSE	0.0000	13.4826
		AIC	Perfect fit	45.5384
Baker-Lonsdale	3/2 × [1 - (1 - F/100)2/3] - F/100 = kBL× t	R2 adjusted	0.9330	0.8418
		RMSE	165.8327	95.2837
		AIC	95.2837	61.7267
Peppas-Sahlin	F = k1 × tm + k2 × t2m	R2 adjusted	1.0000	0.9602
		RMSE	0.0000	23.9593
		AIC	−249.0782	50.7130

Notes: F, Percentage of drug released at time t; k0, Zero order release constant; k1, First order release constant; kH, Hi-guchi release constant; kkP, Release rate constant, and bn, diffusional release exponent; kHC, Release constant relevant to Hixson-Crowell model; kHB, Combined constant corresponding to Hopfenberg model in which kHB = k0/(C0 ×

α0) where k0, erosion rate constant, C0, initial drug concentration in the matrix, α0, initial radius for a slab/cylinder/sphere structure, and n, 1, 2, and 3 for the slab, cylinder, and sphere structure, respectively; kBL, Combined constant related to Baker-Lonsdale model in which kBL = [3 × D × Cs/(r02 × C0) where D, diffusion coefficient, Cs, saturation solubility, r0, initial radius for a sphere/cylinder/slab structure, and C0, initial drug concentration in the matrix; ck1, Constant relevant to the Fickian kinetics, and ck2, constant relevant to Case-II relaxation kinetics, and cm, diffusional release exponent.

Appendix B: Composition of simulated vaginal fluid (SVF) as reported by Owen and Katz for human system [25], with pH adjusted to 7.

Component	Concentration (g/L)
NaCl	3.51
KOH	1.40
Ca(OH)2	0.222
Bovine Serum Albumin	0.018
Lactic Acid	2.00
Acetic Acid	1.00
Glycerol	0.16
Urea	0.4
Glucose	5.0
Porcine mucin type III	15

Appendix C: Data collected on Large White x Landrace sows for optimization of PSVF.

Sow parity	Days until due date	Vaginal pH	Temperature
1	1	7	37.8
1	2	8	38.1
1	0	7	39.1
1	2	7.5	37.7
3	3	7	38.1
4	3	7	37.9
0	3	7.5	38
0	1	7	38.2
2	0	8	37.7
3	0	7.5	37.7
4	0	7	36.5
2	0	6.5	38.2
1	1	7.5	38.2
2	0	7	38
4	0	8	38.5
1	0	7	38
6	2	7.5	38
4	1	6.5	38.1
4	0	7.2	37.5
3	1	6.5	37.6
1	2	7	38.2
1	1	7.5	37.5
4	0	7	37.2
3	1	6.5	38.5
Average		7.1	37.9

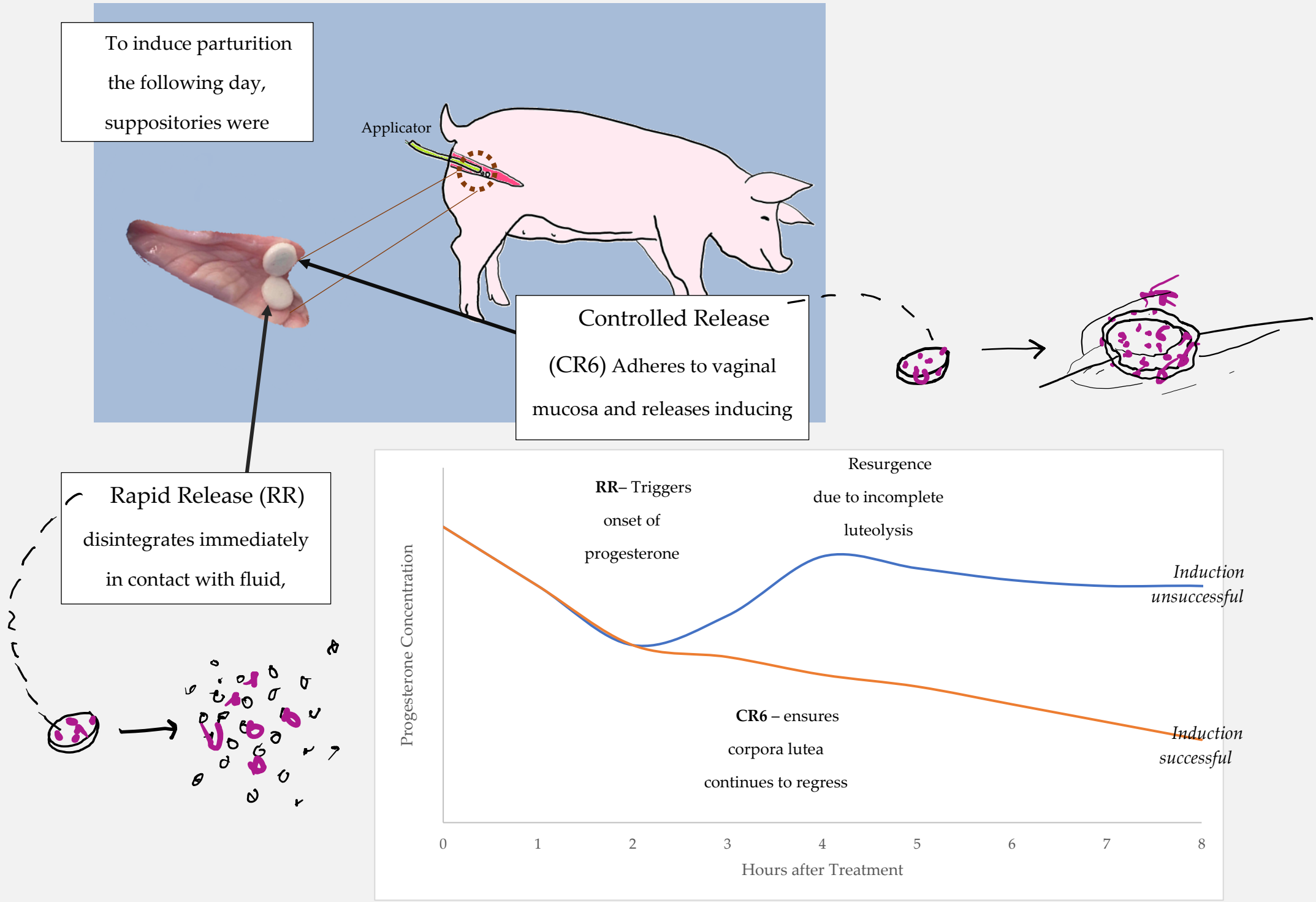
References

1. KilBride, A.; Mendl, M.; Statham, P.; Held, S.; Harris, M.; Cooper, S.; Green, L. A cohort study of preweaning piglet mortality and farrowing accommodation on 112 commercial pig farms in England. *Prev. Vet. Med.* **2012**, *104*, 281-291.
2. Muns, R.; Nuntapaitoon, M.; Tummaruk, P. Non-infectious causes of pre-weaning mortality in piglets. *Livest. Sci.* **2016**, *184*, 46-57.
3. Baxter, E.M.; Edwards, S.A. Piglet mortality and morbidity: Inevitable or unacceptable? In *Advances in Piglet Welfare*; Elsevier: 2018; pp. 73-100.
4. Kaeoket, K. The effect of dose and route of administration of R‐cloprostenol on the parturient response of sows. *Reprod. Domest. Anim* **2006**, *41*, 472-476.
5. Alonso-Spilsbury, M.; Ramirez-Necoechea, R.; Gonzlez-Lozano, M.; Mota-Rojas, D.; Trujillo-Ortega, M. Piglet survival in early lactation: a review. *J. Anim. Vet. Adv.* **2007**.
6. Villanueva-García, D.; Mota-Rojas, D.; Martínez-Burnes, J.; Olmos-Hernández, A.; Mora-Medina, P.; Salmerón, C.; Gómez, J.; Boscato, L.; Gutiérrez-Pérez, O.; Cruz, V. Hypothermia in newly born piglets: Mechanisms of thermoregulation and pathophysiology of death. *J. Anim. Behav. Biometeorol* **2020**, *9*, 0-0.
7. Ward, S.A.; Kirkwood, R.N.; Plush, K.J. Are larger litters a concern for piglet survival or an effectively manageable trait? *Animals* **2020**, *10*, 309.
8. Kirkden, R.; Broom, D.; Andersen, I. Invited review: piglet mortality: management solutions. *J. Anim. Sci.* **2013**, *91*, 3361-3389.
9. Houben, M.A.; Tobias, T.J.; Holstege, M.M. The effect of double nursing, an alternative nursing strategy for the hyper-prolific sow herd, on herd performance. *Porc. Health. Manag.* **2017**, *3*, 1-7.
10. Alexopoulos, J.G.; Lines, D.S.; Hallett, S.; Plush, K.J. A review of success factors for piglet fostering in lactation. *Animals* **2018**, *8*, 38.
11. Vande Pol, K.D.; Tolosa, A.F.; Bautista, R.O.; Willard, N.C.; Gates, R.S.; Shull, C.M.; Brown, C.B.; Alencar, S.A.; Lents, C.A.; Ellis, M. Effects of drying and providing supplemental oxygen to piglets at birth on rectal temperature over the first 24 h after birth. *Transl. Anim. Sci.* **2021**, *5*, txab095.
12. Kirkwood, R.N. Induction of parturition in sows. *Thai. J. Vet. Med.* **2015**, *45*, 487.
13. Kirkwood, R.N.; Aherne, F.X.; Production. Increasing the predictability of cloprostenol-induced farrowing in sows. *J. Swine Health,* **1998**, *6*, 57-59.
14. Decaluwe, R.; Janssens, G.; Declerck, I.; de Kruif, A.; Maes, D. Induction of parturition in the sow. *Vlaams Diergeneeskundig Tijdschrift,* **2012**, *81*, 158-165.
15. Vanderhaeghe, C.; Dewulf, J.; de Kruif, A.; Maes, D. Non-infectious factors associated with stillbirth in pigs: a review. *Anim. Reprod. Sci.* **2013**, *139*, 76-88.
16. Robert, S.; Passille, A.-M.D.; St-Pierre, N.; Pelletier, G.; Petitclerc, D.; Dubreuil, P.; Brazeau, P. Effect of the stress of injections on the serum concentration of cortisol, prolactin, and growth hormone in gilts and lactating sows. *Can. J. Anim. Sci.* **1989**, *69*, 663-672.
17. Ougi, K.; Okada, K.; Leong, K.H.; Hayashi, Y.; Kumada, S.; Onuki, Y. Effect of the molecular mobility of water adsorbed by disintegrants on storage-induced hydrolytic degradation of acetylsalicylic acid incorporated into tablets under humid conditions. *Eur. J. Pharm. Sci.* **2020**, *154*, 105502.
18. Espinoza, R.; Hong, E.; Villafuerte, L. Influence of admixed citric acid on the release profile of pelanserin hydrochloride from HPMC matrix tablets. *Int. J. Pharm.* **2000**, *201*, 165-173.
19. Nokhodchi, A.; Norouzi-Sani, S.; Siahi-Shadbad, M.-R.; Lotfipoor, F.; Saeedi, M. The effect of various surfactants on the release rate of propranolol hydrochloride from hydroxypropylmethylcellulose (HPMC)-Eudragit matrices. *Eur. J. Pharm. Biopharm.* **2002**, *54*, 349-356.
20. Siepmann, J.; Peppas, N.A. Modeling of drug release from delivery systems based on hydroxypropyl methylcellulose (HPMC). *Adv. Drug Deliv. Rev.* **2012**, *64*, 163-174.

21. Caccavo, D.; Lamberti, G.; Barba, A.A.; Abrahmsén-Alami, S.; Viridén, A.; Larsson, A. Effects of HPMC substituent pattern on water up-take, polymer and drug release: An experimental and modelling study. *Int. J. Pharm.* **2017**, *528*, 705-713.
22. Tospitakkul, P.; Kraomkaew, K.; Thammasin, K.; Uttarak, P.; Nuntapaitoon, M.; De Rensis, F.; Tummaruk, P. Induction of parturition by double administration of prostaglandin F2alpha in sows reduces the variation of gestation length without affecting the colostrum yield and piglet performance. *J. Vet. Med. Sci.* **2019**, 18-0725.
23. Kalíková, K.; Tesařová, E.; Bosáková, Z. HPLC method for enantioselective analysis of cloprostenol. *J. Pharm. Biomed. Anal.* **2008**, *46*, 892-897.
24. Bhat, S.; Shivakumar, H. Bioadhesive controlled release clotrimazole vaginal tablets. *Trop. J. Pharma. Res.* **2010**, *9*.
25. Owen, D.H.; Katz, D.F. A vaginal fluid simulant. *Contraception* **1999**, *59*, 91-95.
26. Lorenzen, E.; Follmann, F.; Jungersen, G.; Agerholm, J.S. A review of the human vs. porcine female genital tract and associated immune system in the perspective of using minipigs as a model of human genital Chlamydia infection. *Vet. Res.* **2015**, *46*, 1-13.
27. Chaibva, F.A.; Khamanga, S.M.; Walker, R.B. Swelling, erosion and drug release characteristics of salbutamol sulfate from hydroxypropyl methylcellulose-based matrix tablets. *Drug Dev. Ind. Pharm.* **2010**, *36*, 1497-1510.
28. Berginc, K.; Škalko-Basnet, N.; Basnet, P.; Kristl, A. Development and evaluation of an in vitro vaginal model for assessment of drug's biopharmaceutical properties: Curcumin. *AAPS PharmSciTech* **2012**, *13*, 1045-1053.
29. Hiorth, M.; Nilsen, S.; Tho, I. Bioadhesive mini-tablets for vaginal drug delivery. *Pharmaceutics* **2014**, *6*, 494-511.
30. Ma, Y.; Yang, Y.; Xie, J.; Xu, J.; Yue, P.; Yang, M. Novel nanocrystal-based solid dispersion with high drug loading, enhanced dissolution, and bioavailability of andrographolide. *Int. J. Nanomed.* **2018**, *13*, 3763.
31. Kucera, S.U.; DiNunzio, J.C.; Kaneko, N.; McGinity, J.W. Evaluation of Ceolus™ microcrystalline cellulose grades for the direct compression of enteric-coated pellets. *Drug Dev. Ind. Pharm.* **2012**, *38*, 341-350.
32. Ghimire, M.; Hodges, L.A.; Band, J.; O'Mahony, B.; McInnes, F.J.; Mullen, A.B.; Stevens, H.N. In-vitro and in-vivo erosion profiles of hydroxypropylmethylcellulose (HPMC) matrix tablets. *J. Control. Release* **2010**, *147*, 70-75.
33. Mohamed, F.A.; Roberts, M.; Seton, L.; Ford, J.L.; Levina, M.; Rajabi-Siahboomi, A.R. The influence of HPMC concentration on release of theophylline or hydrocortisone from extended release mini-tablets. *Drug Dev. Ind. Pharm.* **2013**, *39*, 1167-1174.
34. Vueba, M.; Batista de Carvalho, L.; Veiga, F.; Sousa, J.; Pina, M. In vitro release of ketoprofen from hydrophilic matrix tablets containing cellulose polymer mixtures. *Drug Dev. Ind. Pharm.* **2013**, *39*, 1651-1662.
35. Levina, M.; Rajabi-Siahboomi, A.R. An Industrial Perspective on Hydrophilic Matrix Tablets Based on Hyproxypropyl Methylcellulose (Hypromellose). In *Hydrophilic matrix tablets for oral controlled release*; Springer: 2014; pp. 53-85.
36. Gonçalves-Araújo, T.; Rajabi-Siahboomi, A.R.; Caraballo, I. Application of percolation theory in the study of an extended release Verapamil hydrochloride formulation. *Int. J. Pharm.* **2008**, *361*, 112-117.
37. Heng, P.W.S.; Chan, L.W.; Easterbrook, M.G.; Li, X. Investigation of the influence of mean HPMC particle size and number of polymer particles on the release of aspirin from swellable hydrophilic matrix tablets. *J. Control. Release,* **2001**, *76*, 39-49.
38. Zarmpi, P.; Flanagan, T.; Meehan, E.; Mann, J.; Fotaki, N. Biopharmaceutical understanding of excipient variability on drug apparent solubility based on drug physicochemical properties. Case study: superdisintegrants. *AAPS Pharm. Sci. Tech.* **2020**, *22*, 1-17.

39. Kouchak, M.; Mahmoodzadeh, M.; Farrahi, F. Designing of a pH-triggered Carbopol®/HPMC in situ gel for ocular delivery of dorzolamide HCl: in vitro, in vivo, and ex vivo evaluation. *AAPS Pharm. Sci. Tech.* **2019**, *20*, 1-8.
40. Aguilar-de-Leyva, Á.; Campiñez, M.D.; Jost, F.; Gavira, M.; Caraballo, I. Study of the critical points in combined matrix tablets containing both inert and swelling excipients. *J. Drug Deliv. Sci. Technol.* **2019**, *52*, 885-894.
41. Caraballo, I.; Fernández-Arévalo, M.; Holgado, M.; Rabasco, A. Percolation theory: application to the study of the release behaviour from inert matrix systems. *Int. J. Pharm.* **1993**, *96*, 175-181.
42. Viridén, A.; Wittgren, B.; Larsson, A. Investigation of critical polymer properties for polymer release and swelling of HPMC matrix tablets. *Eur. J. Pharm. Sci.* **2009**, *36*, 297-309.
43. Akbari, J.; Saeedi, M.; Enayatifard, R.; Doost, M. Development and evaluation of mucoadhesive chlorhexidine tablet formulations. *Trop. J. Pharm. Res.* **2010**, *9*.
44. Gunvaldsen, R.; Waldner, C.; Harding, J. Effects of farrowing induction on suckling piglet performance. *J. Swine Health Prod.* **2007**, *15*, 84-91.
45. Straw, B.; Bates, R.; May, G.; Production. Influence of method of administration of prostaglandin on farrowing and relationship between gestation length and piglet performance. *J. Swine Health,* **2008**, *16*, 138-143.

Graphical Abstract



Chapter 6: Discussion.

Discussion

At the beginning of this thesis, it was predicted that a certain management strategy could be implemented around farrowing to help improve litter performance. Chapter 1 presented insight into approaches that showed promise, particularly in the area of the peripartum sow. Use of analgesia, alternative farrowing environments and therapeutic anti-inflammatory products led to the hypothesis that the periparturient period is a time in which the sow and piglets are intrinsically linked. The idea of treating sows with a potent analgesic in Chapter 1 was developed in the hopes that relieving sow discomfort would improve subsequent performance of her litter. This in itself was not an original idea, with Chapter 1 giving examples of where anti-inflammatory and analgesic medications were tested on sows around farrowing, but the type and timing of steroid anti-inflammatory drug (SAID) treatment could provide new scientific knowledge. Dexamethasone is an anti-inflammatory steroid, meaning it can pass directly through cell walls and deliver relief to inflamed tissue across multiple sites. Results from Chapter 2 found timing of treatment in relation to parturition had a significant effect on treatment outcome. A risk to an effective treatment outcome was evident following dexamethasone administration one day before farrowing. Sows receiving dexamethasone treatment at this time had piglets with lower birthweights and reduced colostrum intake, and a lower blood protein uptake at 24 h. These unfavorable results lasted to weaning, with piglets from these sows having significantly lower weights at day 18 after dexamethasone administration. In contrast, when the sow received dexamethasone treatment on the day of farrowing, the daily gain of piglets showed slight improvements but with no significant differences in birth and weaning weights. In humans, the use of dexamethasone in late gestation remains controversial for a similar reason [1-2]. Benefits are seen in the survival of preterm babies [3-6], but this may be at the expense of short- and long-term health of the infant [7,8]. Another factor to consider is the long-term health of the mother. In rodent models, treatment of pregnant rats with dexamethasone in late gestation resulted in lower feed intakes and reduced body mass [9]. Although these results had no long-term impact at six months post weaning, the reduced feed intake may have negatively impacted lactation. Conversely, Plush et al. [10] found feed intake was increased when sows were given SAIDs on day 114 of gestation which would have a positive implication on sow body mass. In the current study, no differences were evident in piglet weaning weights, suggesting

dexamethasone did not have significant effects on sow milk yields. With such a potent treatment, a follow up investigation could study the future pregnancy and weaning performances of sows originally treated with dexamethasone to determine whether dexamethasone had any long-term implications. Such an investigation may provide greater insight into whether dexamethasone affects short and/or long-term conditions of the sow in ways that could affect future breeding ability.

One of the key findings of the first investigation was that dexamethasone increased daily weight gain in piglets born to primiparous sows. Dexamethasone is a synthetic glucocorticoid, a steroid hormone that has a multitude of roles in the mammalian body [11]. Glucocorticoids have targeted anti-inflammatory properties, but they also have a significant role in late maturation of the fetus. Considering dexamethasone has the ability to bypass chaperone proteins that usually inhibit influx of corticosteroids, there is a possibility it could have affected the piglet in utero. Gilts are usually physically smaller than sows, and their progeny are susceptible to restricted development [12,13]. Because of this, there is a possibility that dexamethasone was influencing late maturation of an otherwise underdeveloped piglet which resulted in an improvement to piglet daily gain. Whether dexamethasone was influencing the daily gain of piglets through improvements to gilt farrowing/lactation performance or via late fetal programming was inconclusive, which led to the formation of Chapters 3 and 4.

Chapter 3 investigated the younger sow, parity 0 and 1, and the effects of dexamethasone when given on the day of farrowing. Previous investigators identified negligible impacts of analgesia on farrowing and lactation performance [14-16] but most were administered late in the farrowing process or post-partum. It was therefore predicted that treatment timing was going to have a profound effect on pain related behaviours, farrowing performance, and nursing positions over 24 h during and after birth. The hours leading up to the delivery of the first piglet is one that is considered particularly painful [17], so dexamethasone was to be administered prior to this phase. The prediction was that dexamethasone was providing inflammatory relief from discomfort associated with farrowing/lactogenesis and was encouraging more restful behaviours in the primiparous sow. If the majority of dexamethasone-treated sows presented a higher proportion of time on their side 24-h post parturition, the litter could have a greater chance of accessing colostrum for healthy gut formation. Although the first investigation (Chapter 2) did not present any differences in colostrum intake

between dexamethasone treated and control gilts, it could be that more of the litter had a chance to suckle and fewer piglets struggled with weight gain during the nursing phase. Our investigation found these speculations to be inconclusive, as no significant differences were observed in behaviours between dexamethasone-treated and control sows (Chapter 3). The procedure of injecting sows with dexamethasone may have masked any treatment benefits as it could have been triggering acute pain and stress in the gilts in the hours leading up to parturition. In addition, the two intravulval prostaglandin injections administered the day prior to farrowing made some sows nervous around workers and researchers. To overcome possible effects of the injection protocol, dexamethasone was also applied as a mucosal deposition into the vagina. Results of diffusion testing in vitro showed dexamethasone has potential to permeate through porcine vaginal mucosa in a constant manner, albeit slower than intramuscular injection. This test gave evidence that an otherwise injectable treatment can be administered in a way that imparts less stress and/or discomfort on the periparturient sow. Young sows and gilts are known for being particularly restless and stressed leading up to farrowing [18], so it was thought this less invasive administration of treatment would prevent an associated stress response. Although human models have shown dexamethasone had an analgesic effect during labor in conjunction with other analgesics [19-21], no differences were observed in posture changes, aggression, or pain related behaviours in sows. The number of sows used for the behavioral studies were similar to other investigations involving pain behaviours [22, 23] but a larger sample size of predominately primiparous sows should increase the chances of observing restless behaviours, aggressive tendencies or animals with a higher susceptibility to pain during parturition. With little evidence of dexamethasone affecting sow behaviour and lactation processes, it was suggested dexamethasone may be affecting final maturation in the piglet, as outlined in Chapter 4. When a piglet is born significantly underweight (<1.0 kg), they are at increased risk of early mortality and low growth rates to and after weaning [24]. For the investigation in Chapter 4, the focus was on how maternal dexamethasone could possibly improve enteric function in underweight piglets immediately after birth. This prediction was developed on the understanding dexamethasone has been found to increase the survival in preterm human infants [25] but its effects on the gastrointestinal tract was less understood. In our investigation, maternal treatment with dexamethasone had no evident effect on macromolecular

uptake of low or normal birthweight piglets, leading to uncertainty as to whether dexamethasone improved gut performance. With a larger pool of animals, this investigation could be furthered by assessing the responses of piglets that have visual evidence of intrauterine growth restriction. Another approach would be to follow litters of dexamethasone treated gilts through and beyond weaning, comparing the gut morphology of piglets at slaughter, along with backfat depth and hot and cold carcass weights. Such an investigation could provide further insight into the long-term effects of dexamethasone on piglets and whether it is worth pursuing as a treatment.

In all of the investigations, understanding when the sow/gilt was going to farrow was important to timing the treatment. This was highlighted in Chapter 2, which showed the provision of dexamethasone can have either positive or negative implications for piglet performance depending on the time relative to parturition. The uncertainty of treatment timing relative to parturition can be reduced if farrowing induction is practiced. However, many commercial production systems are resistant to the use of injectables for non-medicinal purposes, which is one of the reasons farrowing induction is not a more common practice. In addition, the acute pain and distress associated with injections may outweigh any benefits provided by farrowing induction or an anti-inflammatory treatment such as dexamethasone.

This is why alternate ways to control farrowing onset were sought. In the final study an idea is presented that reflects an evolving attitude towards the original question proposed. Rather than questioning 'what management strategy do we need to improve litter survival' it should be 'how can we make current and future strategies possible to improve litter survival? To reiterate, this thesis was formulated with the idea that a management strategy can be implemented around the periparturient period to improve performance of piglets to weaning. Perhaps not completely surprising a 'golden bullet' strategy or discovery was not found within the scope of these studies. In the review of literature in Chapter 1, a multitude of studies across years of research showed a range of different approaches to this very question. Maybe the reason a single management strategy to improve piglet survival during parturition hasn't been found is because there is no 'golden bullet'. Maybe a combination of different strategies that aid both the farrowing sow and piglets (particularly those at-risk of early mortality) would show improvements to pre-weaning survival. A problem that exists from a number of issues should be approached with a number of strategies.

This is what led to the formulation of a non-injectable induction protocol as presented in Chapter 5. This delivery system would be beneficial for one of two reasons. Firstly, to allow future scientists to research the farrowing process during the day on farms that would consider a non-injectable induction. Secondly, to allow farmers to implement said strategies to improve the performance of the litter. Such a strategy would require very strict record keeping of gestation days, as sows should only be induced no more than two days before their calculated due date [16]. Attention would also need to be placed on possible adverse effects of inducing sows to farrow prior to their due date and whether it outweighs the benefit of any management practice (i.e., it would be beneficial to include non-induced sows as one of the control treatments in such a study).

The concept of an injection-free delivery system shows promise, and areas of improvement could be implemented in future investigations. Combining rapid and controlled release tablets into a single delivery system with a dual layer would provide a more practical application for delivery (both to sow and the person applying the treatment). Pharmacokinetic studies on the passage of cloprostenol through plasma analysis would be ideal for refining dosage of the tablet, something that was considered during formulation and testing in Chapter 5. Considering the size of sows and the small concentration of cloprostenol required to initiate luteolysis, pharmacokinetics could be first tested on a smaller animal model for refinement. In addition, mucosal irritation tests would be important to ensure the tablet does not aggravate sensitive sow vaginal tissue.

Another opportunity would be to use this novel drug delivery system to deliver other hormones/medicines or therapeutics. An example would be to incorporate the hormone GnRH into an immediate and extended release to bring on the onset of estrus in sows, eliminating the need for injectable application and the requirement of subsequent doses. The drug delivery system could also be formulated for use in other animal models (with understanding of unique vaginal pH environments) and with drugs that present a more challenging water solubility (which would adjust the focus of extended release onto tablet matrix erosion rather than matrix swelling).

The formulation of a non-injectable induction protocol may also make investigations on dexamethasone treatment at farrowing on primiparous sows possible on larger scale farms. As previously mentioned, targeting larger populations would help strengthen the power of the study and give greater insight into how this steroid hormone influences

the short- and long-term performance of both sow and piglet. Using injection-free applications for both induction agent and dexamethasone, the behaviours of the sow could be extended to measuring sow feed and water intake (requiring installation of measured feeders), body condition of the sows before and after lactation period and the continued monitoring of litter growth after weaning.

The projects conducted as part of this thesis have led to the understanding that the periparturient period is critical to piglet survival, provided greater insight into how a steroidal anti-inflammatory product will influence the survival and performance of piglets to weaning and has provided a tangible protocol for delivering both inducing agents and dexamethasone treatment to sows without injection, reducing chances of sows getting stressed around the presence of a researcher. It is hoped these studies have satisfied the question rephrased from the initial thesis question: to make current and future strategies **possible** for improving litter survival through the continued development of non-injectable induction and anti-inflammatory alternatives.

References

1. Norman, J.; Shennan, A.; Jacobsson, B.; Stock, S. J.; FIGO Working Group for Preterm Birth, Simpson, J. L.; Bianchi. A.; Munjanja, S.; Maria, V. G., Mol, B. W. FIGO good practice recommendations on the use of prenatal corticosteroids to improve outcomes and minimize harm in babies born preterm. *Int. J. Gynaecol. Obstet.* **2021**, *155*(1), 26-30.
2. McCann-Crosby, B.; Placencia, F.; Adeyemi, O.; Dietrich, J.; Wills, R.; Gunn, S.; Axelrad, M.; Tu, D.; Mann, D.; Karaviti, L.; Sutton, V.R. Challenges in prenatal treatment with dexamethasone. *Pediatr. Endocrinol. Rev.* **2018,** *16(*1), p.186.
3. Jaleel, S.N.A.; Sarojum, B.; Sasidharan, B.; Krishnan, P.G. The incidence and outcome of respiratory distress syndrome in preterm babies in relation to administration of antenatal corticosteroids. *Indian J. Child Health* **2018,** *5*(4), pp.267-271.
4. Rasool, A.; Farooq, U.; Nazir, Q.U.; Durani, H.B. Efficacy of two regimens of dexamethasone for Management of preterm labour: pilot study. *J. Ayub. Med. Coll. Abbottabad* **2017**, *29*(3), pp.393-397.
5. Ho, T.T.H.; Truong, Q.V.; Nguyen, T.K.A.; Le, M.T. Nguyen, V.Q.H. Antenatal dexamethasone use and respiratory distress in late preterm infants: results from first Vietnamese matched cohort study. *BMC pregnancy. childbirth*, **2021**, *21*(1), pp.1-9.
6. Kuk, J.Y.; An, J.J.; Cha, H.H.; Choi, S.J.; Vargas, J.E.; Oh, S.Y.; Roh, C.R.; Kim, J.H., Optimal time interval between a single course of antenatal corticosteroids and delivery for reduction of respiratory distress syndrome in preterm twins. *Am. J. Obstet. Gynecol.* **2013**, *209*(3), pp.256.
7. Qin, G.; Lo, J.W.; Marlow, N.; Calvert, S.A.; Greenough, A.; Peacock, J.L. Postnatal dexamethasone, respiratory and neurodevelopmental outcomes at two years in babies born extremely preterm. *PLoS One* **2017**, *12*(7), e0181176.
8. Hrabalkova, L.; Takahashi, T.; Kemp, M.W.; Stock, S.J. Antenatal corticosteroids for fetal lung maturity-too much of a good thing? *Curr. Pharm. Des.* **2019**, *25*(5), 593-600.

9. Motta, K.; Gomes, P.R.; Sulis, P.M.; Bordin, S.; Rafacho, A., Dexamethasone administration during late gestation has no major impact on lipid metabolism, but reduces newborn survival rate in Wistar rats. *Front. Physiol.* **2018**, 9, p.783.
10. Plush, K. J.; Pluske, J.R.; Lines, D.S.; Ralph, C.C.; Kirkwood. R.N. Meloxicam and Dexamethasone Administration as Anti-Inflammatory Compounds to Sows Prior to Farrowing Does Not Improve Lactation Performance. *Animals* **2021** *11*, 8: 2414.
11. Busada, J.T.; Cidlowski, J.A. Mechanisms of glucocorticoid action during development. Current topics in developmental biology.*Curr. Top. Dev. Biol.* **2017**, *125*, 147-170.
12. Craig, J.R.; Hewitt, R.J.; Muller, T.L.; Cottrell, J.J.; Dunshea, F.R.; Pluske, J.R. Reduced growth performance in gilt progeny is not improved by segregation from sow progeny in the grower–finisher phase. *Animal* **2019**, *13*, 2232-2241
13. Wijesiriwardana, U.A.; Pluske, J.R.; Craig, J.R.; Cottrell, J.J.; Dunshea, F.R. Evaluation of sugarcane-derived polyphenols on the pre-weaning and post-weaning growth of gilt progeny. *Animals* **2020**, *10*, 984.
14. Haussmann, M.; Lay, D.; Buchanan, H.; Hopper, J. Pain Relief in Sows Can Help Reduce Post-Farrowing Activity, Which Could Lead to Reduced Piglet Crushing. *Iowa State University Animal Industry Report* **1999**, *1*.
15. Mainau, E.; Ruiz-De-La-Torre, J.; Dalmau, A.; Salleras, J.; Manteca, X. Effects of meloxicam (Metacam®) on post-farrowing sow behaviour and piglet performance. *Anim.* **2012**, *6*, 494-501.
16. Ison, S.H.; Jarvis, S.; Ashworth, C.J.; Rutherford, K.M. The effect of post-farrowing ketoprofen on sow feed intake, nursing behaviour and piglet performance. *Livest. Sci.* **2017**, *202*, 115-123.
17. Mainau, E.; Manteca, X. Pain and discomfort caused by parturition in cows and sows. *Appl. Anim. Behav. Sci.* **2011**, *135*, 241-251.
18. Harris, M.; Li, Y.; Gonyou, H. Savaging behaviour in gilts and sows. *Can. J. Anim. Sci.* **2003**, *83*, 819-821.
19. Wu, S.; Zhong, C.; Huang, A.; Li, J.; Chen, C.; Yuan, H. Feasibility of epidural injection of ropivacaine and dexamethasone for labor analgesia in women with preeclampsia. *Am. J. Transl. Res.* **2021**, *13*, 7921.
20. Maged, A.M.; Deeb, W.S.; Elbaradie, S.; Elzayat, A.R.; Metwally, A.A.; Hamed, M.; Shaker, A. Comparison of local and intra venous dexamethasone on post operative pain and recovery after caeseream section. A randomized controlled trial. *Taiwan. J. Obstet. Gyneco.* **2018**, *57*, 346-350.
21. Dube, P.; Mitra, S.; Singh, J.; Saroa, R.; Mehra, R. Intravenous dexamethasone as an adjunct to improve labor analgesia: A randomized, double-blinded, placebo controlled clinical trial. *J. Clin. Anesth.* **2017**, *43*, 6-10.
22. Ison, S.H.; Jarvis, S.; Hall, S.A.; Ashworth, C.J.; Rutherford, K. Periparturient behavior and physiology: further insight into the farrowing process for primiparous and multiparous sows. *Front. Vet. Sci.* **2018**, *5*, 122.
23. Nowland, T.L.; van Wettere, W.H.E.J.; Plush, K.J. Allowing sows to farrow unconfined has positive implications for sow and piglet welfare. *Appl. Anim. Behav. Sci.* **2019**, *221*, 104872.
24. Quiniou, N.; Dagorn, J.; Gaudré, D.; Variation of piglets' birth weight and consequences on subsequent performance. *Livest. Prod. Sci.* **2002,** *78*(1), 63-70.
25. Delara, M.; Chauhan, B. F.; Le, M. L.; Abou-Setta, A. M.; Zarychanski, R.; W'tJong, G. Efficacy and safety of pulmonary application of corticosteroids in preterm infants with respiratory distress syndrome: a systematic review and meta-analysis. *Arch. Dis. Child Fetal Neonatal Ed.* **2019**, *104*(2), F137-F144.