NST PART II BBS DISSERTATION

DECLARATION FORM

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I certify that this NST Part II BBS dissertation entitled:

Maternal Undernutrition and Programming of Fertility in Female Offspring of Ruminant Livestock

Supervisor: ALISON FORHEAD

Major/Minor Subject: Physiology, Development and Neuroscience with a minor in Zoology

submitted in partial fulfilment of the Regulations for NST Part II Biological and Biomedical Sciences, is my own original work, and that it does not contain any material that has already been used to any substantial extent for a comparable purpose.

Signed…… L. Kirkwood ……

Date……15-04-2021……...

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Summary

Maternal undernutrition (MU) creates a suboptimal environment for fetal development. This may alter the structure and function of fetal organ systems which only develop during critical windows of pregnancy and early postnatal life. If changes persist into adulthood, the fetus shows 'programmed' phenotypes which may predispose to future disease, a phenomenon first observed in epidemiological studies. Although developmental programming of cardiometabolic disease has been thoroughly investigated, research into programming of fertility is less extensive.

Several studies have shown MU to decrease ovulation and pregnancy rates and delay puberty in female offspring in sheep and cattle. This window of MU coincides with formation of the ovarian reserve, folliculogenesis and organisation of connections in the hypothalamic-pituitary-ovarian (HPO) axis in the fetus. This dissertation explores the possible mechanisms underlying programming of fertility in offspring by MU.

Firstly, MU depletes fetal ovarian reserve by decreasing oogonial proliferation and upregulating atresia, however it is unclear how ovarian reserve size affects fertility. The adult ovarian reserve, indicated by antral follicle counts, is reduced in offspring by MU and consequently lowers pregnancy, and ovulation rates. The HPO axis which regulates folliculogenesis and ovulation rate may not be significantly affected by MU, however data for ruminants are limited and confounded by variations in the timing and duration of MU. Timing of puberty appears to be related to birth weight, catch-up growth and adiposity at puberty. Late MU typically decreases birth weight and if undernutrition is continued into the postnatal period, catch-up growth may be limited, delaying puberty. Leptin bridges the reproductive system with the nutritional status of the animal and possibly mediates changes in age at puberty due to MU. The compromised reproductive function in sheep and cattle has implications for the productivity of the livestock industry and places emphasis on adequate maternal nutrition during pregnancy.

Introduction

The impact of a sub-optimal intrauterine environment on various long-term phenotypes of the offspring is well-established. Key epidemiological studies by Barker (1995) revealed correlations between low birth weight and metabolic disease in adulthood, and his work gave rise to the 'Developmental Origins of Health and Disease' hypothesis. The programming phenomenon depicts how a suboptimal intrauterine and early postnatal environment generates persistent phenotypes in the adult offspring due to changes in structure and function of organs as they develop during particular critical windows.

Many studies have investigated the programming of metabolic and cardiovascular disease by manipulating maternal diet in animal models, including rodents and sheep (Lakshmy, 2013). Investigations into the developmental origins of cardiometabolic disease have been driven by its potential importance in human medicine relating to the causes, treatment and prevention of cardiovascular disease and type II diabetes which have a high prevalence and mortality rate (McMillen and Robinson, 2005). In contrast, a smaller field of research has been dedicated to the programming of fertility. MU may prioritise available nutrients to allocate them to key organ systems for survival, thereby compromising reproductive function. This is of particular importance in livestock species where fertility determines the ease of conception, number of offspring, and ultimately the yield in the meat industry. Likewise, in the dairy industry, the rapid succession of pregnancies maximises milk yield by continuing lactation.

Objectives

Overall, this dissertation aims to examine the developmental programming of female fertility in cattle and sheep, by hypocaloric maternal undernutrition (MU), and the potential contributing mechanisms.

The specific objectives are:

- To analyse the impact of MU on ovarian function reflected by ovulation and pregnancy rates
- To investigate possible mechanisms of programming ovarian function which include:
    - Depletion of fetal ovarian reserve
    - Reduction in adult antral follicle count
    - Alterations to hypothalamic-pituitary-ovarian (HPO) axis activity and gonadotropin concentrations
- To analyse the impact of MU on the timing of puberty
- To investigate possible mechanisms of programming the age at puberty which include:
    - Low birth weight and catch-up growth
    - The role of leptin as a permissive cue in the onset of puberty

Programming of Ovarian Function by MU

Several studies have examined the impact of MU during gestation on various indicators of offspring fertility, defined as the ability to conceive and subsequently give birth to live offspring. It is commonly measured by ovulation and pregnancy rates. Prenatal nutrition must be sufficient to support the establishment of gonads and neuroendocrine connections in the hypothalamus which regulate ovarian function, thus MU may hinder prenatal developmental processes to influence future fertility.

Maternal Diet and Ovulation and Pregnancy Rates in Offspring

Ovulation rate is defined as the number of oocytes released at ovulation during one oestrous cycle and can be inferred by measuring the number of corpora lutea by laparoscopy. It defines the maximal possible conception events and thus litter size. It is also a measure of ovarian and

hypothalamic-pituitary function, whereby oocytes are released in response to the gonadotropins, luteinising hormone (LH) and follicle stimulating hormone (FSH).

Studies have shown that MU can influence offspring ovulation rate. Lambs born to ewes whose diet was restricted to 50% of the control diet between 0 and 95 days gestational age (dGA) had a lower ovulation rate at 20 months of age (Rae et al., 2002). This is typically when domesticated ewes first become pregnant to ensure they lamb at two years old. Hence, ovulation rate at 20 months is relevant to determine fertility in the context of sheep farming. In addition, the results were independent of body condition scores at 20 months and circulating gonadotropin profiles.

Furthermore, offspring from ewes which did not receive a supplemented feed and were instead solely grass-fed from 45dGA until term, had fewer multiple births implying a lower ovulation rate (Gunn et al., 1995). Interestingly, ovulation rate was not significantly different between offspring from mothers on either diet, calculated by the number of corpora lutea at post-mortem, 3 weeks after the first breeding season. This suggests that maternal nutrition influenced offspring litter sizes by a mechanism other than ovulation rate such as embryo survival. This contrasting effect may be explained by the different windows of MU, whereby ovulation rate was impacted by MU in early but not late gestation. Notably ovulation rate can be influenced by extrinsic factors other than maternal diet such as photoperiod (predominantly sheep), stress, age, postnatal diet and intrinsic factors such as breed genetics, body condition score and neuroendocrine feedback systems (Scaramuzzi et al., 2011). Additionally, there is evidence to suggest that although ovulation rate may vary between sheep, this has no effect on pregnancy rate, and therefore may not be a valid measurement of fertility (Schoenian and Burfening, 1990).

Pregnancy rate can also infer female fertility, defined as the proportion of successful pregnancies that carry the fetus(es) until term out of all the animals which have been mated. It allows evaluation of many processes which occur during pregnancy which may impact a successful pregnancy from being achieved. Much like ovulation rate, MU has been shown to negatively impact pregnancy rates in

offspring. In a group of seven lambs born to mothers who experienced 50% diet restriction between 28 and 78dGA, just one produced a lamb at 2 years old. This was in comparison to the control group where all 7 offspring produced a live lamb (Long et al., 2010). This was concordant with lower progesterone profiles during the oestrous cycle in the MU offspring. These lower pregnancy rates could be explained by the association of low periovulatory progesterone concentrations with lower embryo survival (Ashworth et al., 1989). The study by Long *et al.* (2010) was limited by a small sample size, however, which may affect the reliability of results in a larger herd.

Similarly, in cattle, offspring from mothers without protein supplementation during gestation had a 21% lower pregnancy rate, and pregnancy at first service was half as successful compared to offspring of mothers on a supplemented diet (Martin et al., 2007). Protein supplementation may support placental function to maximise transfer of available nutrients to the fetus for development of the reproductive system. Supplementation during late pregnancy may however also improve lactation and the postnatal diet. This has previously been shown to increase multiple births, but does not appear to affect the pregnancy rate in sheep (Rhind et al., 1998). Further studies might research how isocaloric micronutrient maternal restriction affects offspring fertility to reveal more specific dietary components which contribute to programming mechanisms seen in global MU models. For instance, maternal isocaloric selenium supplementation decreased fetal follicle proliferation in sheep (Grazul-Bilska et al., 2009).

In contrast, other studies found MU had no significant impact on the pregnancy rate or calving interval in cattle (Mossa *et al.*, 2009; Cushman *et al.*, 2014). This may be explained by the milder dietary restrictions in these studies of 60% and 75% respectively, which may have been insufficient to cause programming effects. Although several studies use the diet recommended by the National Research Council (2001, 2007) for pregnant cattle and sheep as a control diet, there is inconsistency in the description of diet composition between studies, sometimes loosely defined as maintenance requirements or simply *ad libitum*. This may make it harder to draw valid comparisons between

studies. Overall, ovulation and pregnancy rates in offspring is sensitive to MU possibly resulting in fewer multiple births and a higher rate of barrenness.

MU and Fetal Ovarian Development

Prenatal nutrition can affect the development of the ovaries and the numbers of oocytes established during fetal life. Oogonia, the mitotic form of the female gamete, exist during fetal but not postnatal life. This means a fixed number of oocytes exist in the offspring ovary at birth, establishing a non-replenishable reserve available for folliculogenesis and ovulation from puberty onwards. This may impact on the future fertility of the offspring.

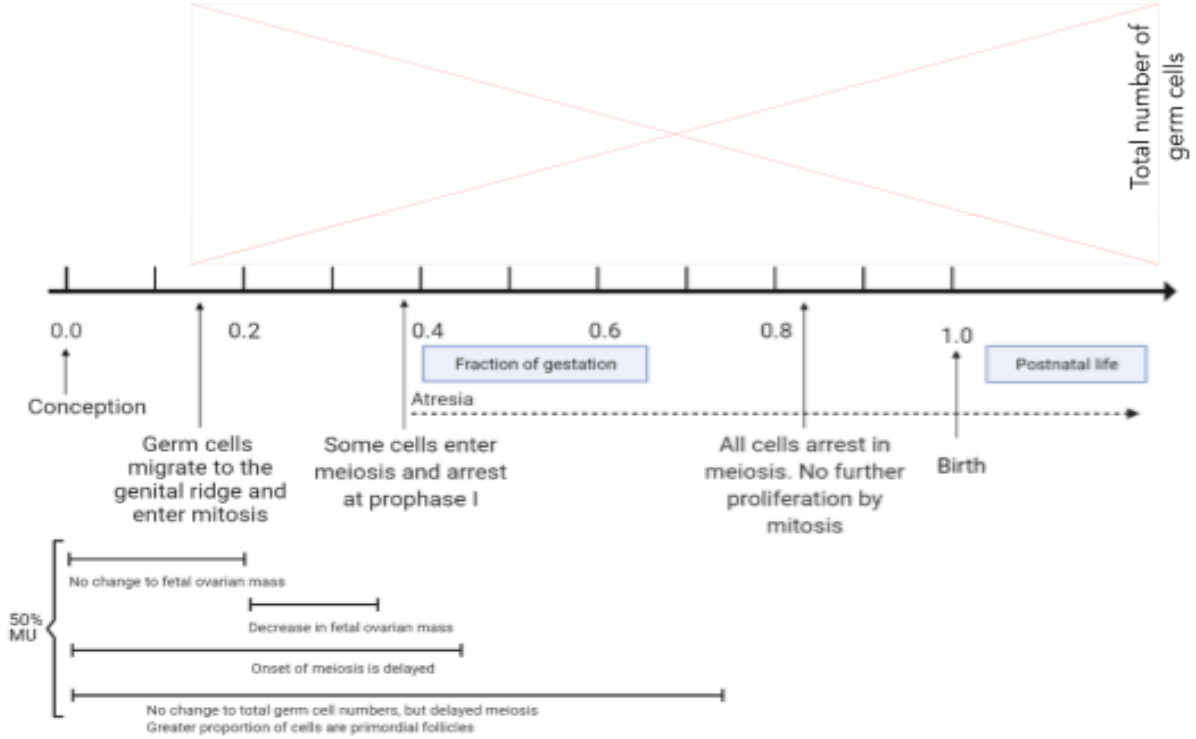


*Figure SEQ Figure * ARABIC 1: Timeline of the development of the fetal ovary in cattle and sheep and the number of germ cells present in the ovaries throughout gestation in cattle. Original diagram by Lucy Kirkwood.*

Blue line: Number of germ cells in the fetal ovary in cattle, data from ADDIN ZOTERO_ITEM CSL_CITATION {"citationID":"Y8xjAUI1","properties":{"formattedCitation":"(Erickson, 1966b)","plainCitation":"(Erickson, 1966b)","noteIndex":0},"citationItems":[{"id":773,"uris":["http://zotero.org/users/7008554/items/V3Z23899"],"uri":["http://zotero.org/users/7008554/items/V3Z23899"],"itemData":{"id":773,"type":"article-journal","abstract":"<section class=\"abstract\"><h2 class=\"abstractTitle text-title my-1\" id=\"d401e2\">Summary.</h2> Qualitative observations of forty-nine bovine foetuses taken at varying stages of gestation revealed the following: (1) the germinal ridge is established at the 32nd day of gestation; (2) due to the formation of the tunica albuginea of the primitive testis, foetal sex is distinguishable by Day 39; (3) meiosis begins in ovaries at 75 to 80 days post coitum; (4) oogonial mitosis is discontinued by 150 to 170 days; (5) the majority of the oocytes have attained their stage of rest (pachytene) and are enveloped in primordial follicles by Day 170; and (6) vesicular follicles appear in ovaries at Day 250 of gestation. Total germ-cell numbers, based on

Figure 1 summarises the timeline of development of the fetal ovary in sheep and cattle. When adjusted by gestation lengths (sheep 145 days, cattle 285 days average), events in sheep occur at a similar fraction of gestation (G) as cattle (Smith et al., 2014).

The rate of mitosis of oogonia during fetal life is critical in determining the number of germ cells which populate the ovary as they cannot divide after birth. A 50% diet restriction in sheep between 30 and 50dGA, coinciding with the greatest rate of oogonial proliferation, reduced the ovarian mass in sheep at 50dGA, whereas diet restriction before the proliferative phase did not change ovarian mass at 30dGA (Figure 1; Rae *et al.*, 2001). They did not report how ovarian mass or germ cell number changed by term, however, and changes in proliferation may be reversed by atresia, follicular degeneration, that follows.

To further investigate the mechanism underlying ovarian reserve dynamics, proliferation specifically in germ cells was quantified in histological studies using the marker Ki67. In response to 60% MU between 50 and 135dGA in sheep, a decrease in proliferating oogonia was observed in their growth-restricted fetuses at 135dGA. It may however be more relevant for MU exposure and observations of proliferation rates to be taken when proliferation is most dynamic between 0.2 to 0.6G since mitosis of germ cells occurs at a relatively low rate in late gestation (Figure 1).

In a contrasting model, ewes which were fed *ad libitum* compared to a control diet from conception, had lower placental weights and consequently growth-restricted fetuses with reduced follicle counts at 103dGA and 131dGA (Da Silva et al., 2002, 2003). Although nutrients were more abundant in the maternal circulation, it is possible their transfer to the fetus was limited by the smaller placenta. It therefore supports the previous study whereby limitation of nutrient delivery and growth restriction of the fetus causes a reduction in follicle count.

Surprisingly, in sheep, imposing 50% MU from conception increased germ cell numbers by 62dGA compared to control diets (Borwick et al., 1997). However, there was no mention how the difference in germ cell counts at 62dGA affected the total ovarian reserve at birth. This is important as

subsequent germ number depletion is significant and happens over a large window and may reverse any differences observed early in gestation.

One explanation for the increase induced by early MU may be due to a delay in the onset of meiosis and continuation of mitosis, since the observation at 62dGA coincides with the onset of meiosis at 0.43G (Figure 1). In fetal sheep where maternal nutrition was halved between conception and 65dGA, fewer cells were resting in meiosis compared to control fetuses (Figure 1; Rae *et al.*, 2001). This study found that although meiosis was delayed, it did not impact on the total number of germ cells at 65dGA, contradicting results by Borwick *et al.* (1997). In addition, MU impaired follicular maturation as a greater proportion remained as primordial follicles, whereas a smaller proportion formed primary, secondary or antral follicles. Nevertheless, it is unknown how this impacts adult ovarian function as the primordial follicles will eventually mature postnatally. Secondly, the results by Borwick *et al.* (1997) may be explained by a delay in atresia onset which depletes total ovarian reserve. However, the authors failed to quantify apoptosis at this time point and thus further investigations are required to determine whether the onset of atresia is affected by MU.

Atresia may be upregulated instead of delayed by MU, and since this continues throughout life, may have repercussions for the ovarian reserve in the long term. In sheep, the expression of Bax, a pro-apoptotic gene, was upregulated in 110dGA fetal ovaries when 50% MU was imposed from conception to 110dGA (Lea et al., 2006). Furthermore, there was evidence of increased oxidative stress in oogonial DNA at 78dGA as a result of 50% MU which may trigger upregulation of atresia (Murdoch et al., 2003). The cause of the oxidative stress may provide further information on the molecular mechanisms of the effects of MU. Despite this, there was no report of apoptotic bodies or consequences for the total germ cell number in either study. This evidence is supported by rodent models whereby MU increased follicular atresia which subsequently lowered oocyte count in fetal ovaries at 0.7G (Wang *et al.*, 2018).

Ovarian development is also aided by the growth of surrounding connective and vascular tissues. It may be the case that stromal tissue proliferation was disproportionately affected by MU compared to germ cells to reduce ovarian weight observed by Rae *et al.* (2001). In sheep, the apoptotic gene Bax was upregulated in the ovarian vasculature at 110dGA when challenged with MU between 0-110dGA or as early as 0-30dGA (Lea et al., 2006). This evidence indicates that ovarian vasculature development may be restricted by MU and this could explain the reduced capacity of the oogonia to proliferate and the delay in developmental stages of fetal ovaries, for example due to the lack of growth factors or oxygen delivery. Indeed, any changes seen in the rate of atresia may be directly due to MU or as a result of apoptosis of supporting somatic cells and may be the focus of future research.

Implications of Fetal Ovarian Reserve for Future Fertility

It is evident that fetal ovarian development and germ cell counts are affected by MU, particularly during early pregnancy. However, the extent to which these responses to MU have consequences for germ cell numbers in later life and resultant fertility remains to be established. In cattle, follicle number begins to decrease at around 5 years of age (Figure 2; Erickson, 1966a). Although this coincides with a decline in pregnancy rates from 5 years old (Cushman et al., 2009), a decrease in fertility is unlikely to be caused by the depletion of the ovarian reserve as there are still over 1000 primordial follicles present and around 100 growing follicles at 15-20 years. These older follicles and oocytes, however, may be of poorer quality or have altered endocrine capacity which may affect ovarian function and thus fertility, independent of ovarian reserve size. Animals born with lower follicle numbers as a result of MU may reach an ovarian reserve associated with older cattle sooner, prematurely ageing the ovary. The decline in ovarian reserve and fertility with a large inter-species range of follicle numbers is difficult to assess and has not been investigated in detail in response to MU.

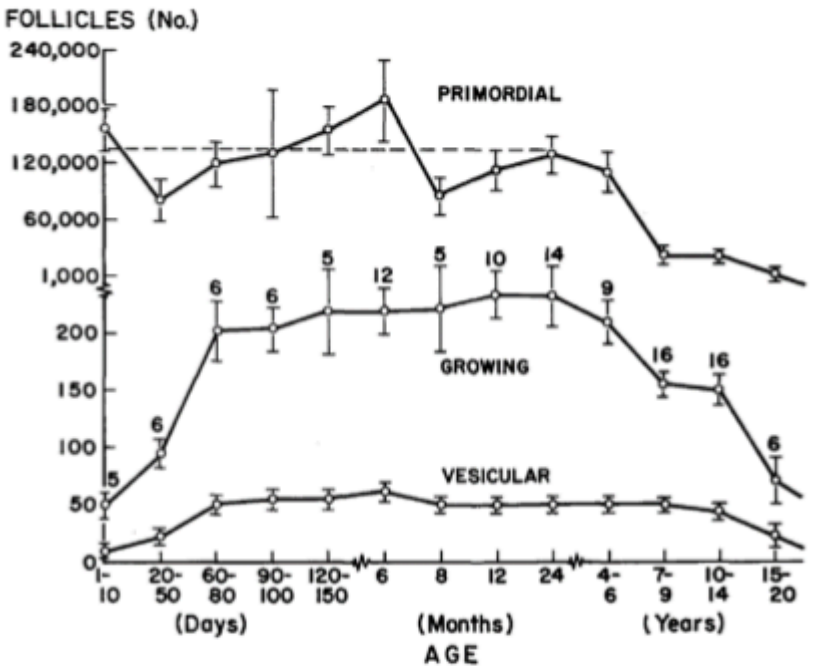


Figure 2: Follicle numbers in cattle over the first two years of life. The dashed line represents an average number of primordial follicles (~135,000 follicles) in the first 2 years of life in cattle. Graph from Erickson (1966a).

Furthermore, while MU evidently impacts the fertility of their immediate offspring, these effects could extend to the subsequent generation. Germ cells which give rise to grand-progeny form in the fetus and may be susceptible to acquisition of epigenetic marks on genes relevant to fertility. Understanding the transgenerational effects of MU on offspring fertility would require epigenome-wide association studies to examine epigenetic marks on genes associated with germ cell quality and compare these between fertile and sub-fertile animals.

MU and Antral Follicle Count in the Adult Offspring

Whilst MU affects aspects of fetal ovarian development, it is also important to study the effects on subsequent adult ovarian reserve and function. Programmed effects may only become obvious after puberty once ovulation begins, or changes observed in the ovaries at birth may be corrected before puberty with little consequence for fertility. The antral follicle count (AFC) may better reflect the effects of MU on ovarian function in adult offspring rather than total follicle count which can be very large with significant variation between individuals. This is performed by sonography and is a

measure of the number of follicles >3mm in diameter. Although some antral follicles are present at birth, they are stimulated by FSH and so are more numerous in the adult ovary.

Heifers with a high AFC have a greater total number of oocytes (Figure 3; Ireland *et al.*, 2008). AFC is therefore an appropriate way to estimate the ovarian reserve in adulthood, particularly since this observation was at 10-14 months old which is relevant to the first breeding season in heifers. The distribution of follicle stages in the adult ovaries were similar in the low versus high AFC groups. It appears that the smaller proportion of secondary and tertiary follicles observed in the fetal ovaries may be corrected by adulthood with limited implications for fertility. Low AFC has direct consequences for fertility as it is associated with low circulating progesterone concentrations, pregnancy rates and ovulation rates in cattle (Jimenez-Krassel *et al.*, 2009; Mossa *et al.*, 2012; Cushman *et al.*, 2000). Heifers with low AFC are also less likely to give birth in the first 21 days of the breeding season, an indication of lower conception rates (McNeel and Cushman, 2015).

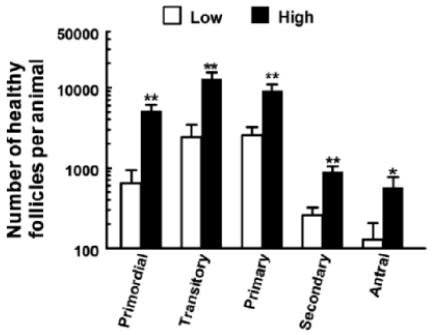


*Figure 3: Numbers of healthy follicles in heifers with low and high AFC for each stage of folliculogenesis in heifers aged 10-14 months old. The clear bars represent heifers which have a low AFC and the black bars represent heifers with a high AFC. Taken from Ireland et al. (2008). *P<0.05 and **P<0.01.*

Furthermore, low AFC is correlated with low body and ovarian weights at birth, suggesting that there is a prenatal mechanism which predisposes heifers to subsequent changes in AFC (Cushman et al., 2009). The effect of MU on AFC has been investigated in bovine offspring from prepubertal age, 7 and 18 weeks, to breeding age, at 14 months (Table 1). Where dietary restriction was imposed during early pregnancy, coinciding with the proliferative phase of follicle production, AFC was depleted

(Mossa et al., 2013), whereas nutrient restriction later in gestation or alterations to protein intake

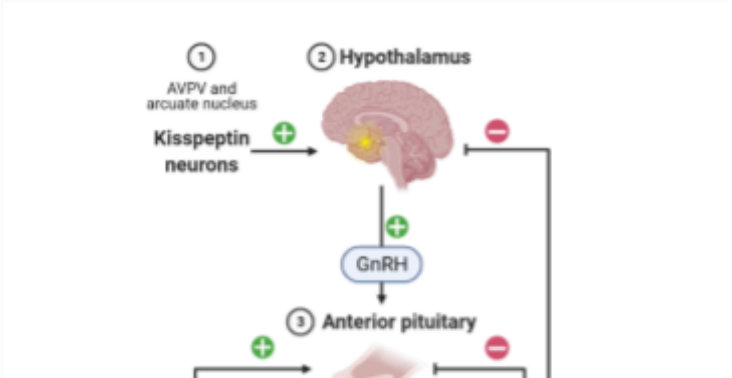
*Table SEQ Table * ARABIC 1: The effect of MU on offspring AFC at various ages in cattle. Average gestation length in cattle is 285 days.*

alone had no effect (Cushman et al., 2014; Sullivan et al., 2009). The timing of MU is therefore important for the resultant programming effect. Despite several investigations into the molecular mechanisms by which MU affects fetal ovarian reserve, it is not clear if the same mechanisms cause variation in AFC as there is opportunity for it to be affected further in the postnatal period. Data for sheep are scarce, presumably since AFC is easier to measure in cattle, although similar mechanisms of follicle maturation are at play in both species.

Study	Maternal diet restriction (% of control diet)	Window of undernutrition (days of gestation)	Age of offspring at time of observation (months)	Findings
Mossa *et al.*, 2013	60%	-11-110	1.6	40% ↓in mean AFC
			4.1	38% ↓in mean AFC
Cushman *et al.*, 2014	75%	95-285	14	No change to ovarian reserve
Mossa *et al.*, 2013	60%	-11-110	13	20% ↓ in mean AFC
			20	33% ↓in mean AFC
Sullivan *et al.*, 2009	30% protein intake	0-93	6 and 23	No change to AFC
		93-181		No change to AFC

MU and Hypothalamic-Pituitary Control of Ovarian Function in Offspring

Maternal nutrition may affect ovarian function in the offspring by altering the activity of the reproductive neuroendocrine axis, involving the hypothalamus, pituitary and ovaries (Figure 4; HPO



axis). If neural connections in the brain are disrupted during formation in the fetus, the hormones which regulate ovarian function may be dysregulated in later life. This may create a persistent change to the reproductive axis to give rise to a programmed effect. Low gonadotrophin concentrations may hinder fertility, given their positive effect on folliculogenesis, reflected by AFC, and control of ovulation.

Deligeorgis *et al.* (1996) found 60% MU resulted in a reduced LH secretion in 55-day old female lambs in response to exogenous GnRH injection. The nutritional challenge was imposed from 30dGA to term, coinciding with the period when the HPO axis connections are established and so perhaps prevents these hypothalamic connections from forming properly (Figure 5). Nevertheless, at 55 days old, it is unclear how this difference may affect fertility in later life or whether the altered LH profile is significant enough to affect ovarian function.

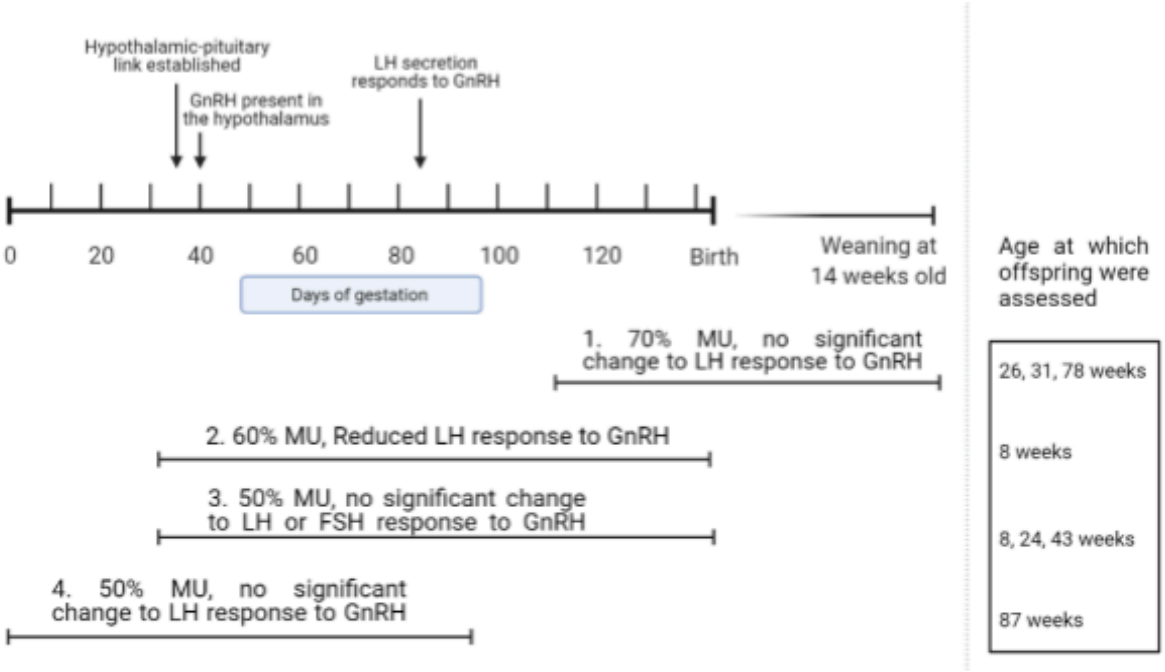


Figure 5: Summary of studies examining the effect of MU on the HPO activity of offspring in sheep. Diagram made by Lucy Kirkwood. Timeline uses information from Deligeorgis et al. (1996) and Rhind et al. (2001).

- *Borwick et al. (2003)*
- *Deligeorgis et al. (1996)*
- *Kotsampasi et al. (2009)*
4. *Rae et al. (2002)*

To examine the effect of MU on negative feedback from the steroid hormones, MU and control offspring were ovariectomised to remove the source of the steroid hormones (Borwick et al., 2003). This had no effect on circulating gonadotrophin profiles between control and 70% restricted maternal diets. This was also the only study to examine oestrogen, GnRH, LH and FSH receptor expression at the pituitary, yet they found no significant differences between either group by pubertal age, at 78 weeks. More data is required for studying this aspect of the HPO axis, such as performing the study at different ages or with different severities of MU. Furthermore, local control by inhibin may alter FSH profiles and the effect of MU on inhibin concentrations could also be investigated. Furthermore, steroid hormones are detectable in the fetus as follicles form in cattle, but whether they have any effects on follicle or HPO axis formation at these early stages of development is unknown (Fortune et al., 2013). These studies may reveal potential alterations to HPO activity and feedback loops in response to MU.

Three other experiments found that there were no significant changes in gonadotrophin profiles in lambs which experienced prenatal or postnatal undernutrition (Figure 5). These experiments examined circulating gonadotrophin concentrations following GnRH injection in older lambs ranging from 2 to 20 months. The lambs are sexually mature at this age, making it more relevant to forming conclusions about fertility. Furthermore, ovulation rate was significantly lower in lambs born to undernourished mothers without changes to LH secretion or birth weight (Rae et al., 2002). Also, pituitary gonadotrophin secretion did not differ between cattle with low or high AFC of various ages (Mossa et al., 2010). This suggests that programming effects on fertility are independent of pituitary gonadotrophin secretion regulated by the HPO axis.

There may be compensatory mechanisms that overcome programming which occurs *in utero* so that changes observed at 55 days (Deligeorgis *et al.* 1996) are no longer significant by 20 months of age (Rae et al., 2002). It may also be that undernutrition during this particular developmental window determines the extent of the programming of the HPO axis, particularly during the period of hypothalamic neuron formation. However, Kotsampasi *et al.* (2009) studied identical periods of MU and offspring age as Deligeorgis *et al.* (1996) and found offspring HPO axis responsiveness unchanged. Although sample sizes varied between studies, the reasons responsible for differential outcomes are ultimately unknown and require further investigation.

Deligeorgis *et al.* (1996) found that lambs were born significantly lighter in response to MU, and yet there was no significant difference in birth weights in all the studies where MU had no effect on GnRH responsiveness in offspring (Borwick et al., 2003; Kotsampasi et al., 2009; Rae et al., 2002). This suggests that programming of the HPO axis by MU may relate to changes in fetal growth. Fetal growth-restriction may have consequences for postnatal metabolism and catch-up growth to drive the programming effect. However, by reproductive age, programming of offspring fertility by MU appears to be independent of changes in the responsiveness of the HPO axis.

MU and Onset of Puberty

Puberty is marked by oestrus behaviour, the onset of regular oestrous cycles and ovulation in females. This occurs between 8-10 months in cattle (Perry, 2016) and at 7-8 months in sheep, although this is largely determined by the photoperiod (Redmond et al., 2011a). The transition is gradual and requires a convergence of metabolic and endocrine signals, attainment of a critical body weight and activation of the HPO axis (Figure 4).

The transition period at puberty involves a decrease in the sensitivity of the negative feedback loop exerted by oestrogen. This is mediated by downregulation in oestrogen receptors at the hypothalamus and pituitary (Perry, 2016). In addition, hypothalamic kisspeptin neuron activity activates GnRH neuron activity which increases the frequency and magnitude of pulsatile gonadotropin release from the pituitary. Peripubertal sheep show upregulated Kiss1 mRNA in the hypothalamus, suggesting that it has a key role in activating the HPO axis at puberty (Redmond *et al.*, 2011*a*). Also, intravenous kisspeptin injection stimulated pulsatile LH release to induce ovulation in prepubertal sheep (Redmond *et al.*, 2011*b*) and in adult sheep during seasonal anoestrous (Caraty et al., 2007). This in turn triggers follicle development, oestrogen production and eventually ovulation.

Imposing MU across the window when the hypothalamic circuitry of the HPO axis is forming may affect subsequent activation at puberty, mediated by kisspeptin and GnRH neurons (Figures 4 and 5). However, changes to the onset of puberty by MU may be indirect through changes in birth weight and subsequent catch-up growth before puberty, given the importance of attaining a critical weight for puberty to occur. Nutrition in late gestation is particularly important in determining birth weight since growth is more rapid than during early gestation when organogenesis mostly occurs.

Heifers which experienced 65% MU 100 days before birth entered puberty on average 19 days later than those born to well-fed mothers, although this result was just outside statistical significance (Corah et al., 1975). There was a significant weight difference in the mothers after 100 days prepartum diet restriction, likely due to the negative energy balance as a result of rapid fetal growth

in the second half of pregnancy. Furthermore, the weaning weight of the MU offspring was significantly lighter. This suggests milk yield may have been poor in early postnatal life whilst mothers recovered once they returned to control diets after pregnancy, although milk quality and quantity was not defined in this study. Early postnatal diet is particularly important for catch-up growth and consequently, onset of puberty, as heifers which were fed well from 7 to 12 months old gained more weight and entered puberty significantly earlier those under-fed (Short and Bellows, 1971). These findings could be supplemented with cross-foster studies, whereby the prenatal diet can be controlled independently to the postnatal diet to investigate the influence of each on the timing of puberty.

In sheep, placental and fetal growth restriction caused by maternal overnutrition for the entirety of gestation decreased birth weights by 1.5kg and offspring continued to be lighter at puberty compared to control offspring, suggesting poor catch-up growth. As a result, puberty was significantly delayed in males although there was no effect in females (Da Silva *et al.*, 2001). In contrast, there was no significant difference in the age at puberty with early or late MU in cattle (Mossa *et al.*, 2013; Cushman *et al.*, 2014) or sheep (Kotsampasi et al., 2009). This may be explained by the lack of effect of MU on weight at birth and puberty between groups in both studies.

Puberty is advanced in several species in response to rapid catch-up growth when recovering from a low birth weight (Yao et al., 2020). In a model of maternal overnutrition in rodents, birth weight was lower, followed by rapid catch-up growth and this advanced puberty in offspring (Sloboda et al., 2009). On the other hand, the timing of puberty in females was significantly delayed when mothers were undernourished during both the prenatal and postnatal period, but not the prenatal period alone (Léonhardt et al., 2003). It is possible that the postnatal diet may limit catch-up growth to delay puberty. However, rodents are altricial species whereby hypothalamic maturation occurs in the first 3 weeks of postnatal life (Bouret and Simerly, 2007). Ruminants, however, are precocial species, and the hypothalamus is structurally and functionally mature by late gestation (Mühlhäusler et al.,

2004). This may explain the different impacts that pre- and postnatal undernutrition have on hypothalamic control of puberty in each species. Overall, it is likely that the onset of puberty is only delayed in studies where both birth weight and subsequent catch-up growth were restricted in response to MU.

Control of Puberty by Leptin

There is substantial crosstalk of signals between the nutritional and reproductive centres in the hypothalamus to link the onset of puberty to birth weight and catch-up growth as a result of MU. The peptide hormone leptin acts both to regulate appetite and as a permissive factor for the onset of puberty in the hypothalamus (Amstalden et al., 2014). It is synthesised in adipose tissue and plasma concentrations reflect the adiposity and energy reserves of the animal. In mice, intravenous injection of leptin advanced puberty in mice (Ahima et al., 1997). Selective deletion of the leptin receptor using Cre-lox from GnRH neurons (Quennell et al., 2009) or kisspeptin neurons (Donato et al., 2011), however, did not affect the advancement of puberty when exogenous leptin was administered. This suggests leptin acts upstream to these neurons, more specifically at the ventral pre-mammillary nucleus as selective re-expression of the leptin receptor in this area of the hypothalamus in receptor-deficient mice restored the advancement of puberty by leptin injection (Donato et al., 2011). Whether leptin acts in the same location in the hypothalamus in ruminants is yet to be established.

Although exogenous leptin administration does not advance puberty in fed ruminants, it has effects on the reproductive axis during nutritional deficits (Zieba et al., 2005). Sheep which were lean and underfed were also hypogonadic with low Kiss1 expression in the arcuate nucleus of the hypothalamus, and intravenous leptin injection was able to increase Kiss1 mRNA and LH secretion to improve reproductive function (Backholer *et al.*, 2010; Henry *et al.*, 2001). Offspring in a state of nutritional deficit at birth due to MU may be sensitised to the advancement of puberty by leptin.

Greater adiposity and therefore circulating leptin may additionally be caused by rapid catch-up growth following MU, further accelerating the onset of puberty.

Changes to Postnatal Leptin Surge and Hypothalamic Organisation

In rodents, the postnatal leptin surge is important for organising and guiding neurons in the hypothalamus (Bouret et al., 2004). MU and subsequent catch-up growth resulted in higher leptin concentrations yet heavier offspring than control offspring, implying some leptin resistance in rats (Iwasa et al., 2010). This study did not examine STAT3 signalling in leptin-sensitive neurons in the hypothalamus to confirm this, however. Consequently, hypothalamic Kiss1 mRNA was low, delaying puberty by 2 days as a result. Leptin signalling could be a possible mechanism by which MU programmes changes in the control of age at puberty in early postnatal life.

The postnatal leptin surge, which occurs at 6 to 9 days old in lambs (Long et al., 2011), may have a less significant impact on the ovine hypothalamus since it is significantly more mature at birth compared to rodents. In cattle, birth weight and postnatal leptin concentrations were lowered by MU in the final 100 days of pregnancy (LeMaster et al., 2017). Furthermore, a delayed postnatal leptin surge in sheep induced by maternal overnutrition has been linked to subsequent adult obesity, suggesting that the surge impacts on future appetite regulation controlled by the hypothalamus (Long et al., 2011). Intriguingly, glucocorticoid concentrations were raised in both these studies, although evidence relating to the postnatal leptin surge and glucocorticoid concentrations in response to specifically MU is scarce for ruminants. Additional experiments could explore the ability of glucocorticoids to affect neuron organisation in the hypothalamus relating to both appetite and reproduction and how this changes with MU.

Leptin primarily has its actions on neurons in appetite centres such as pro-opiomelanocortin, neuropeptide Y and agouti-related protein neurons of the arcuate nucleus which indirectly affect reproductive neurons (Figure 6). For example, leptin acts to inhibit NPY neurons which subsequently have inhibitory actions on LH secretion in sheep (Barker-Gibb et al., 1995). Therefore, it is possible

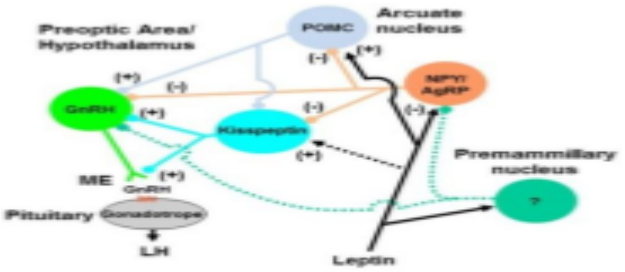


Figure 6: Interactions between hypothalamic neurons which control the reproductive axis. The action of leptin on the reproductive axis at the ventral premammillary nucleus has been established in mice (Donato et al. 2011), although its pathways to the GnRH and kisspeptin neurons are yet to be defined, indicated by the green dashed lines. POMC = Pro-opiomelanocortin, NPY = neuropeptide Y, AgRP = agouti-related protein. Diagram taken from ADDIN ZOTERO_ITEM CSL_CITATION {"citationID":"fup5YegY","properties":{"formattedCitation":"(Amstalden et al., 2011)","plainCitation":"(Amstalden et al., 2011)","noteIndex":0},"citationItems":[{"id":1607,"uris":["http://zotero.org/users/7008554/items/VLKESMC5"],"uri":["http://zotero.org/users/7008554/items/VLKESMC5"],"itemData":{"id":1607,"type":"article-journal","abstract":"The pubertal process is characterized by an activation of physiological events within the hypothalamic-adenohypophyseal–gonadal axis which culminate in reproductive competence. Excessive weight gain and adiposity during the juvenile period is associated with accelerated onset of puberty in females. The mechanisms and pathways by which excess energy balance advances puberty are unclear, but appear to involve an early escape from estradiol negative feedback and early initiation of high-frequency episodic gonadotropin-releasing hormone (GnRH) secretion. Hypothalamic neurons, particularly neuropeptide Y and proopiomelanocortin neurons are likely important components of the pathway sensing and transmitting metabolic information to the control of GnRH secretion. Kisspeptin neurons may also have a role as effector neurons integrating metabolic and gonadal steroid feedback effects on GnRH secretion at the time of puberty. Recent studies indicate that leptin-responsive neurons within the ventral premammillary

that changes to appetite-related neurons due to a delayed postnatal leptin surge may result in altered reproductive function and possibly the timing of puberty. Further studies are required to investigate the actions of leptin in the preoptic area controlling reproductive function in the fetal and new-born ruminant hypothalamus in the context of MU.

The fetus is informed about the availability of nutrients it requires for postnatal growth before it is born. Therefore, programming mechanisms may have evolved to generate advantageous changes in the fetus to best adapt to the anticipated environment after birth. For example, programming mechanisms in response to MU may alter the metabolism of the offspring to optimise nutrient use or upregulate appetite to compensate and promote adequate postnatal growth with the nutrition available. When nutrient availability is plentiful postnatally, this may lead to rapid catch-up growth, unmasking the programming effects. Similarly, offspring who continue to be undernourished in early life may delay puberty to allow more time to reach the critical weight. However, in the event of rapid catch-up growth, the timing of puberty may be accelerated. This could also allow offspring to extend their reproductive lifetime to produce more offspring once nutrient availability is restored to levels which can support a growing population.

Implications for the Livestock Industry

Fertility is important in the livestock industry to increase the herd size and maintain high productivity. This may be negatively impacted by MU which decreases ovulation and pregnancy rates and if undernutrition is continued postnatally, delays puberty. The differences in reproductive physiology and animal management in sheep and cattle means that the effects of MU have different implications for livestock industries, be that meat or dairy.

Sheep

Sheep are primarily reared for lamb, although in some instances may be milked too. Multiple births as a result of high ovulation rate are desirable in sheep which typically have 1-3 lambs. However, in the event of singleton births possibly due to low ovulation rate, cross-fostering can redistribute triplet lambs from other ewes to optimise maternal milk supply per lamb. It is however important for the ovulation rate to be above 1 to ensure regular ovulation to increase the chances of pregnancy during the mating season. Sheep experience seasonal anoestrus and so if pregnancy is unsuccessful in the autumn, the ewe will be barren for the entire year, lowering parity and thus lifetime yield. Moreover, lambs are sent to market before their reproductive ability is known. If it transpires that a lamb has poor fertility, it may be culled and sold as mutton. This is not as valuable as lamb and may have consequences for profits.

The timing of puberty onset is not particularly relevant to sheep as fertility is largely dictated by the photoperiod. That said, lambs can become fertile in the first autumn after they are born if born early enough in spring and this can improve profitability. However, it is important that sheep reach a critical weight (~60% mature body weight) at breeding as this strongly influences reproductive success (Gaskins et al., 2005). Furthermore, mating ewe lambs any earlier may cause a conflict between growth of the fetus and the immature ewe and may limit future productivity.

Cattle

Unlike sheep, the fertility of cattle is unrelated to seasons and so cattle can enter puberty anytime during the year. This makes advancement of puberty more relevant in cattle than sheep to extend their reproductive lifespan. If heifers can reproduce sooner, this potentially increases parity and subsequent reproductive endurance to increase yield and reduce demand of replacement heifers. However, in many instances, puberty is advanced only by a few days and may not significantly affect the lifetime productivity of the cattle. Cattle are also polyoestrous, meaning they have several opportunities throughout the year to conceive. With every cycle where pregnancy is unsuccessful, there is a 21-day delay until the next attempt. In dairy cows, milk yield continues to deteriorate with every unsuccessful attempt at pregnancy and thus impacts yield and financial gain. This is not as important in beef cattle which do not have the same financial consequences if conception is unsuccessful. Lowered pregnancy rates resulting from MU may mean that cows require more services to become pregnant. This can be a significant economic cost if performed artificially with sexed semen, which is common practice in the dairy industry to increase the chances of female offspring to replace the herd and continue producing milk.

Application to Animal Management

Given MU negatively impacts ovulation and pregnancy rates, it is important that nutrition on farms is optimised for the pregnant livestock to avoid culling animals with low fertility. In the interest of offspring fertility, adequate nutrition early in gestation is just as important as late in gestation when growth and energy demands increase exponentially. It is important, however, to avoid overnutrition as this has also been shown to have consequences for offspring fertility (Da Silva et al., 2003). Manipulating the diet of the herd may be an effective way of improving the fertility of the following generation and could supplement existing techniques used in the livestock industry. The extent to which the reproductive consequences of MU affect the economy of the livestock industry has not yet

been quantified. This would help to determine whether techniques used to mitigate suboptimal fertility by MU are profitable or whether other techniques are more cost-effective.

Conclusion

Overall, MU provides a suboptimal environment for growth and development of the fetus and can lead to permanent changes in the structure and function of the reproductive system. This may predispose the offspring to subfertility from birth, thus programming the future ability of the offspring to reproduce. Effects of MU on the reproductive system can be observed in the fetus but might also only emerge after puberty. This dissertation has shown that MU has negative consequences for the fertility of female offspring through a series of mechanisms (Figure 7).

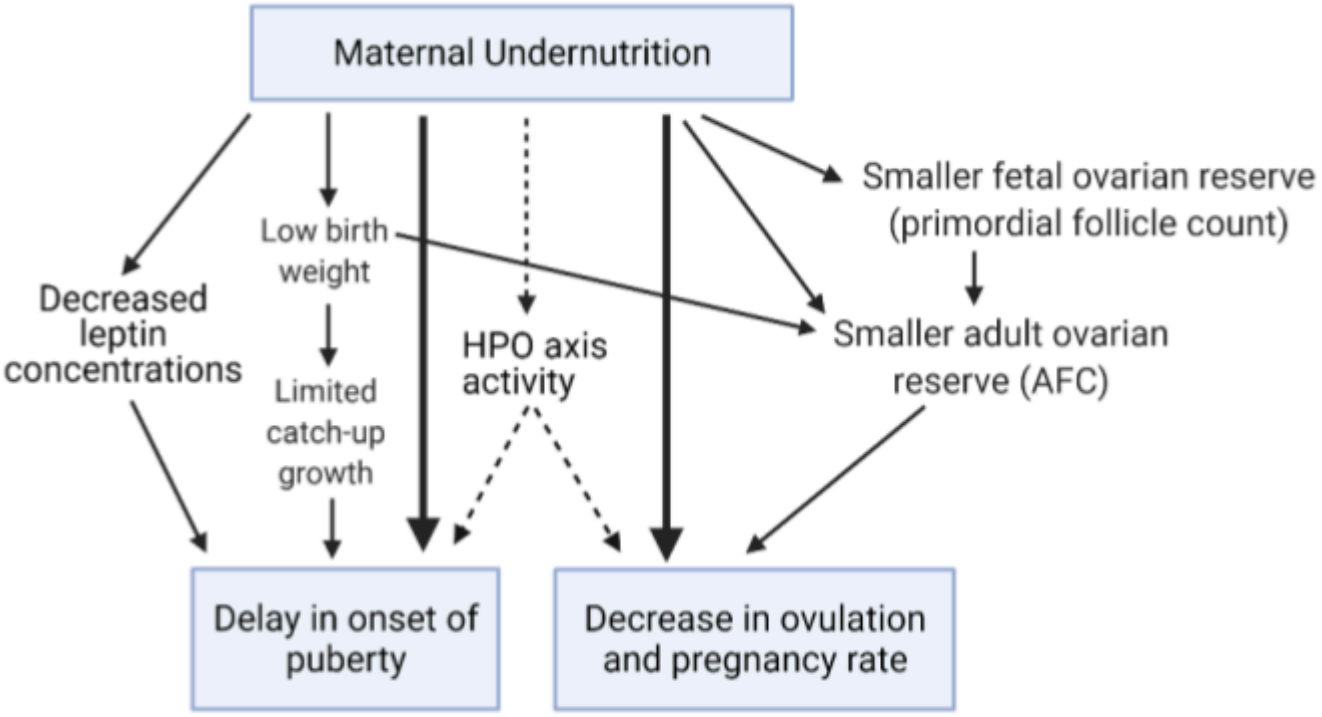


Figure 7: Summary of the possible mechanisms whereby MU influences offspring fertility. Dotted line indicates a weaker link. Diagram by Lucy Kirkwood.

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