Bovine Oestrus Behaviour, Hormones, Physiology and Manipulation

A good knowledge of the bovine oestrus cycle underpins successful management of cattle farming enterprises as so much of bovine production is dependent on ensuring cows produce a calf every year. As with many domestic species, the oestrus cycle is orchestrated by the release of hormones from the ovary, hypothalamus and anterior pituitary gland. This article will explain the role of the relevant hormones and how they interact to coordinate the normal reproductive behaviour in female bovines.

Bovine Oestrus Behaviour:

Recognising oestrus behaviour is crucial in many dairy cow enterprises as in the UK the vast majority of dairy farms use AI (Artificial Insemination) to achieve conception. Without the bull to detect the signs displayed by the cow indicating she is receptive to mating, the managers of dairy herds must recognise these behaviours to know when the optimal timing for AI will be.

Oestrus behaviours are initiated by the increasing levels of oestrogen that the pre-ovulatory dominant follicle on the ovary releases. There are a series of secondary oestrus signs that are seen both before and after the primary oestrus sign. These include; rubbing, bunting, sniffing (esp. anogenital area of other cows), licking, chin-resting, increased activity and mounting other cows. These secondary signs are frequently observed in the preceding 12 and following 18 hours after oestrus. It is important to note that the expression of these behaviour signs is very dependent on the environment suitability.

Primary oestrus behaviour in the cow is standing to be mounted by other cows. This is why the phrase 'standing heat' is often used to describe when the cow is showing the primary oestrus behaviour. Primary oestrus behaviour can occur over a 12 hour period during which time the cow is receptive to the bull. However, in high-yielding dairy cows the signs of primary oestrus may only be shown for a total of 6 minutes for each oestrus cycle, making frequent observation of cows for detection of heat vitally important. The surge in Luteinising Hormone (LH) release occurs at the time of standing heat. Ovulation occurs approximately 36 hours after the LH surge.

Summary of Bovine Reproductive Hormones:

1.) GnRH (Gonadotropin releasing hormone): produced by the hypothalamus. Low frequency GnRH pulsatile release will trigger FSH production from the anterior pituitary gland. High frequency GnRH pulsatile release stimulates LH release (following directly the pattern of the GnRH release i.e. a pulse of GnRH triggers a pulse of LH).
2.) FSH (Follicle stimulating hormone): produced by the anterior pituitary gland. FSH recruits a number of small ovarian follicles and begins stimulating their maturation in preparation for ovulation.
3.) LH (Luteinising hormone): produced by the anterior pituitary gland. During follicle maturation the follicles develop LH receptors and their final stages of maturation become LH dependant. The character of the pulsatile LH release will determine the physiological outcome: high frequency low amplitude = follicle emergence, low frequency high amplitude = follicle selection, large amplitude and high frequency (LH surge) = ovulation trigger.
4.) Oestrogen: produced by the developing follicles on the ovary. The main oestrogen produced in the bovine is estradiol (E_2). During the oestrus cycle oestrogen production increases with follicle development producing a positive feedback effect increasing FSH release from the anterior pituitary gland which will support continued maturation of the follicles.
5.) Progesterone: produced by the corpus luteum (CL) which forms following ovulation of the dominant follicle. Progesterone exerts a negative feedback effect on the hypothalamus reducing the release of GnRH. By limiting GnRH release this prevents an LH surge occurring and thus, while progesterone is released no ovulation can occur.
6.) Inhibin: produced by the maturing follicle on the ovary. Functions to suppress the maturation of other follicles on the ovary by a negative feedback effect on the release of FSH on the anterior pituitary gland. There is no negative feedback action on LH release. The reduction in

FSH release will cause the support for the other immature follicles on the ovary to be lost and these will then undergo atresia.

7.) $PGF2_{\alpha}$: Released by the endometrium of the uterus. Prostaglandin $F2_{\alpha}$ is not a hormone but is a chemical messenger that has an integral role in the oestrus cycle. Its function is to induce luteolysis (the disintegration of the corpus luteum) in the absence of an embryo in the uterus. This then causes a decline in progesterone production from the CL. Oxytocin is responsible for stimulating the pulsatile release of $PGF2_{\alpha}$ which is transported in the uterine vein from the endometrium to the ovary where it can exert its effect.

The Bovine Oestrus Cycle:

Now that we have considered the oestrus-associated hormones, and the tissues in the body that are responsible for producing them, we now can now appreciate how these will interact to produce the regular patterns of follicle development and oestrus in the cow. Bovine ovarian activity is in the form of consecutive cycles or 'waves' of follicle recruitment, development, selection, atresia and potential ovulation. All of these processes are coordinated by the hormones discussed above.

It is important to note that there are differences in the oestrus cycle depending on the type of bovine – i.e. is this a dairy cow or is this a heifer (dairy or beef)/beef suckler cow?
Regardless of the type of bovine there are some common features of the oestrus cycle:

- Non-seasonal polyoestrus
- Puberty (onset of ovarian activity) occurs at 65% adult body weight
- (Standard) Oestrus length = 21 days
- Luteal phase = day 0 (ovulation) – day 16 (luteolysis)
- Follicular phase = day 17- day 21
- Progesterone dominates in the luteal phase and oestrogens in the follicular phase
- Ovulation occurs approximately 36 hours after the LH surge

The endocrinology of follicular waves:

- Follicles in the earliest stages of development (termed pre-antral follicles) are not hormone dependant and so develop under the influence of locally produced growth factors in the post-pubertal animal. Once these follicles reach the tertiary stage of development (becoming antral follicles) their continued development is dependent on FSH.
- Recruitment stage: Follicular waves are initiated by an increase in the circulating Follicle Stimulating Hormone (FSH) concentration. The increase in FSH will support the continued maturation of the subset of ovarian follicles that have reached the antral stage (approximately 20 follicles). In effect, the cohort of tertiary follicles have been recruited to the process of synchronous follicle maturation which depends on FSH.
- Development of Follicles: FSH in the systemic circulation continues to drive the maturation of the cohort of follicles. The follicles produce inhibin which will suppress further high-level FSH production ensuring that other pre-antral follicles are not recruited and so follicle development only occurs in discrete waves.
- Dominant Follicle Selection: During this phase one follicle is selected of the cohort to continue the process of maturation, this is the dominant follicle. The exact physiological mechanisms that cause one follicle to be selected from the recruited cohort is not well understood. Research has shown that the selected follicle has a greater propensity to produce estradiol and other molecules that increase its sensitivity to FSH and LH; this is assumed to play a role. The dominant follicle develops receptors for LH but the other (subordinate) follicles do not develop LH receptors. Around this time plasma FSH levels decline and LH plasma levels begin to rise. The declining FSH levels are due to negative feedback exerted by the increasing estradiol release from the dominant follicle. Consequently, the subordinate follicles lose their hormonal support to continue developing and they will become

atretic. Conversely, the selected dominant follicle with LH receptors will continue to be supported as plasma LH concentrations rise. Effectively, the selected dominant follicle releases estradiol in order to cut off the vital supply of FSH to the subordinate follicles which then regress (undergo atresia).

- Maturation and Fate of the Dominant Follicle: The dominant follicle continues to develop maturing to the pre-ovulatory stage under the influence of LH (and basal FSH). During this phase it will release increasing quantities of estradiol, under the influence of LH pulse pattern.
    - If this follicular wave occurs in the luteal phase of the oestrus cycle (i.e. there is a CL present, the first 16 days of the oestrus cycle) the high plasma progesterone concentrations will exert a negative feedback effect on the hypothalamus limiting release of LH. Consequently, no LH surge can occur and ovulation is not triggered. The dominant follicle therefore becomes atretic and regresses.
    - However, if this follicular wave has occurred in the follicular phase of the oestrus cycle where plasma progesterone levels are basal, there is no suppression of LH release. Consequently, as the mature dominant follicle releases maximal levels of estradiol this has a positive feedback effect on the hypothalamus causing massive release of GnRH triggering the LH surge. In response to the LH surge the dominant follicle ovulates releasing the oocyte (and follicular fluid).

Maternal Recognition of Pregnancy:

The cow will enter into another oestrus cycle if a pregnancy is not detected. It is therefore important that if an embryo has been formed that it can signal its presence to the dam to prevent the next oestrus cycle from occurring which would require the loss of the CL that is currently supporting its maintenance in the uterus.

First we will consider what occurs when insemination and fertilisation has not occurred: as the next dominant follicle of the following follicular wave emerges, is selected and matures, the plasma level of estradiol begins to rise. In response to estradiol, endometrial cells upregulate their expression of oxytocin receptors. Oxytocin binding to its receptors on endometrial cells triggers the pulsatile release of $PGF2_{\alpha}$. This is conveyed by the uterine vein to the ovary where it induces luteolysis at day 16 of the oestrus cycle (16 days after ovulation occurred). This ceases progesterone production by the CL and lifts inhibition of GnRH secretion in preparation for the next ovulation to occur and the cycle to repeat.

If fertilisation has occurred and a blastocyst (embryo) has formed: The embryo releases Interferon tau (also referred to as bTP-1 bovine trophoblast protein -1). Interferon tau causes the downregulation of endometrial oxytocin receptors so that the endometrium sensitivity to oxytocin is reduced. Consequently, the oxytocin-mediated release of $PGF2_{\alpha}$ from the endometrium is reduced and this will prevent luteolysis, so the CL is maintained supporting the pregnancy through progesterone production. The embryo has effectively signalled its presence to the dam by interferon tau release.

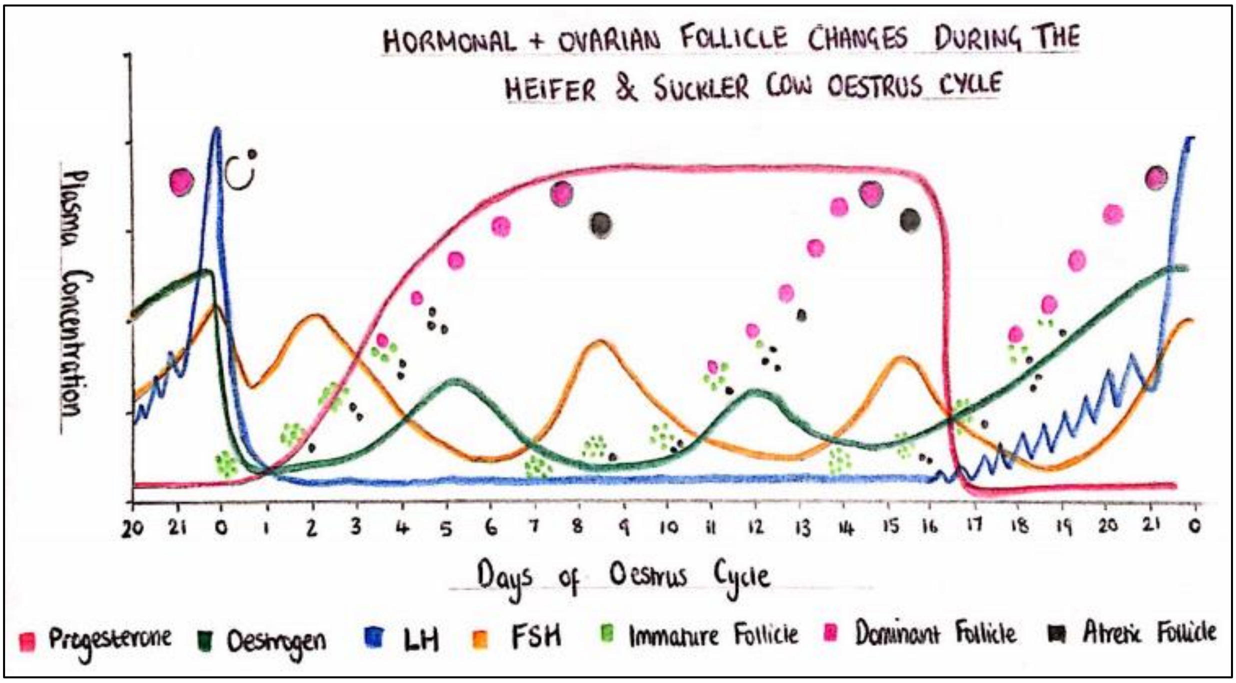
Early embryonic loss can occur due to failure of sufficient interferon tau release and failure of maternal recognition of pregnancy. Smaller embryos are less able to signal their presence to the dam as they release lower levels of interferon tau. Progesterone from the CL supports the growth of the early embryo. Hence, to minimise early embryonic loss, maximising embryonic growth and interferon tau production are essential. Delayed or limited progesterone production by the CL must be avoided to ensure the embryo has sufficient signalling capacity.

It is important to note that the CL is only mature and sensitive to the effects of $PGF2_{\alpha}$ after day 5. This is of relevance when manipulating oestrus cycles as iatrogenic administration of $PGF2_{\alpha}$ between days 0-4 after ovulation will not induce luteolysis.

The Bovine Oestrus Cycle: All heifers and beef (suckler) cows

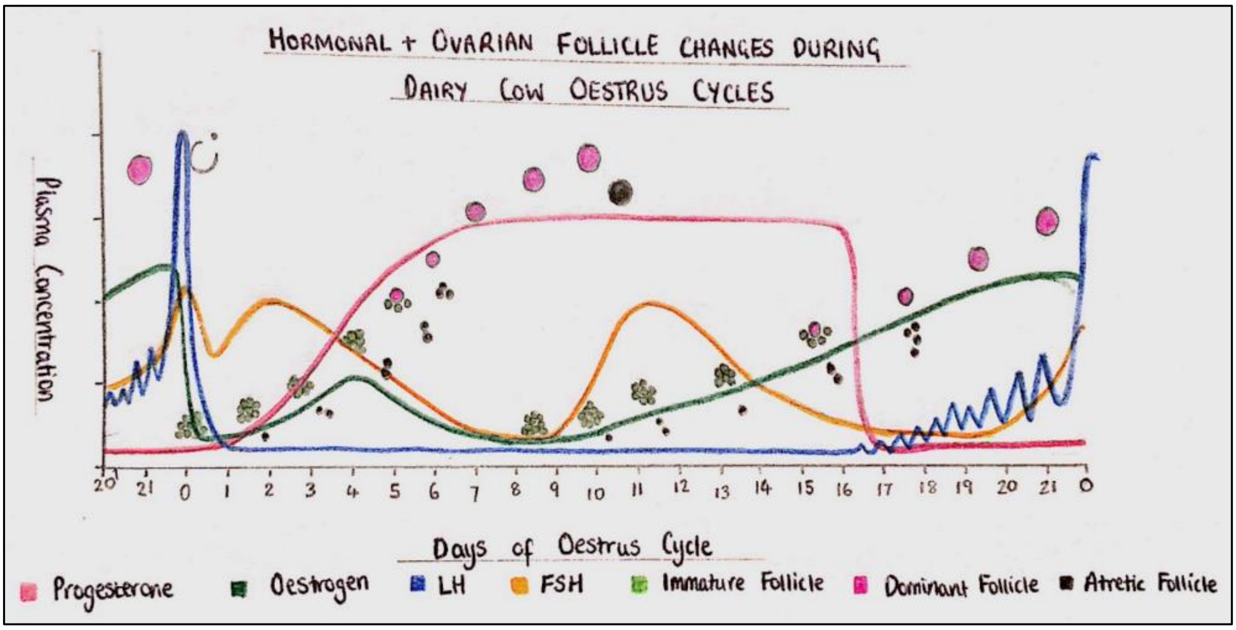
During the 21 day oestrus cycle there are 3 follicular waves, each lasting approximately 7 days. The third wave is the ovulatory wave, the previous two being anovulatory follicular waves

The diagram below illustrates the pattern of follicular development and the associated hormone fluctuations;



The Bovine Oestrus Cycle: Dairy cows

One must remember that dairy cows are the athletes of the bovine world and that they are in significant metabolic stress due to the demands of lactation. This is especially true of early lactation which is the time when we are asking them to return to ovarian activity after parturition and establish pregnancy again. For this reason their oestrus cycles do differ to bovines that are not under the same metabolic stress and one of the key differences is that dairy cows typically show only 2 follicular waves per oestrus cycle, each lasting approximately 10 days in duration. The diagram below illustrates the pattern of follicular development and the associated hormone fluctuations occurring in the dairy cow ovary;



Post-partum Resumption of Ovarian Cyclicity (ROC)

During late gestation the plasma levels of progesterone (from the CL and placenta) and oestrogens (from the feotoplacental unit) are maximal and this exerts a profound negative feedback effect on the hypothalamus. Consequently, from 20-25 days pre-partum, the release of FSH and LH is transiently paused. Hence, follicular waves are no longer initiated (which have persisted through gestation to this point). At parturition plasma levels of progesterone and oestrogens fall rapidly to basal levels, removing the negative feedback effect on the hypothalamus allowing GnRH release to initiate FSH and LH release again. Increasing FSH production will initiate the first post-partum follicular wave. The fate of the resulting dominant follicle is dependent on LH pulse frequency. Hence, ROC is controlled by LH pulse frequency and any factors that influence this will also affect onset of ROC.

Factors affecting LH pulse frequency:

Dairy Cows	Beef Cows
Negative Energy Balance (NEB)	Calf presence (maternal bond)
BCS at calving	Calf suckling
Dry Matter Intake	Negative Energy Balance (NEB)
Health status	BCS at calving
	Health status
	Season (autumn calvers have earlier ROC than spring calvers)

The majority (50-80%) of dairy cows will ovulate their first dominant follicle which develops approximately 10 days post-partum. If cows are in a high NEB status and/or suffering from disease they are less likely to ovulate the first dominant follicle and it will become atretic.

Beef cows do not ovulate their first dominant follicle which also develops approximately 10 days post-partum. This is due to the influence of the calf's presence and its suckling supressing LH release. Beef cows in ideal BCS and good health status typically ovulate their 3rd dominant follicle, around 20 days post-partum. Poor BCS and health status will inhibit ovulation and so these cows may only ovulate their 10th dominant follicle post-partum.

In both beef and dairy cows the first ovulation produces a 'silent heat' – there are no overt signs of oestrus seen. This is because there was not the prior 'progesterone priming' as there was not a CL present immediately before it was ovulated. However, after this first ovulation a CL is produced and hence, the second ovulation post-partum will be associated with typical oestrus signs. The first CL post-partum lasts for a shorter duration and hence the cows will have their next ovulation at a shorter interval than the standard 21 day oestrus cycle. This is termed a 'short cycle'

<u>Manipulation of the Bovine Oestrus Cycle:</u>

Manipulation of the bovine oestrus cycle is an area of livestock veterinary work that herd managers are frequently turning to in order to boost productivity of the herd. This is true of dairy enterprises especially but also in some suckler herd systems. In a simplistic sense, for either dairy or beef enterprises, the key to profitability is ensuring that each cow produces a calf every 365 days. There are of course many other factors such as live-weight gain of beef calves and milk volume and quality that will also influence productivity but ensuring that cows calve down at yearly intervals underpins most bovine enterprises. It is for this reason that manipulation of the bovine oestrus cycle, to boost fertility, is such a valuable tool when managing commercial herds.

Before this article discusses some examples of oestrus manipulation, it is important to realise that chemical manipulation of the oestrus cycle to improve fertility is not a substitute for correcting suboptimal management (environment or stockmanship). As vets we should actively seek to encourage our farmers to critically evaluate the management of their stock to improve heat detection rates, maximise oestrus expression and minimise negative energy balance. This is of primary relevance to dairy herds.

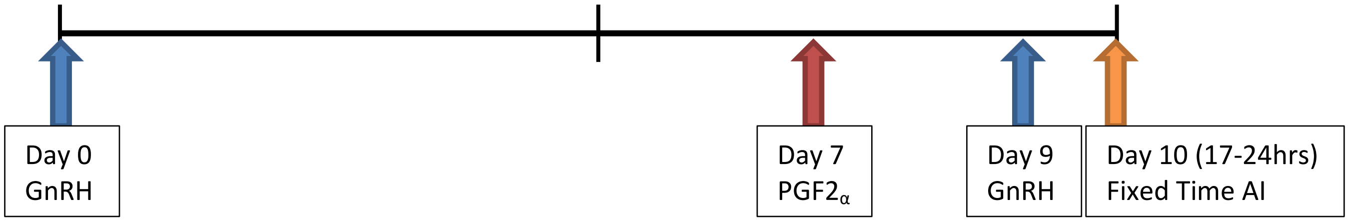
There are many different protocols that are used to manipulate the bovine oestrus cycle and the choice of protocol will depend on; the purpose for manipulation, the ovarian activity of the cows and the farm managers or reproductive advisor's experience. This article cannot discuss all possible protocols and iterations of these but hopes to outline several widely-used options. In order to understand the mechanisms and rationale for the various manipulation programs a sound understanding of the hormonal interactions that occur during the bovine oestrus cycle is essential. This will allow you to understand and logically think through why each pharmaceutical is used and the timing of its use.

Manipulation Protocols: Dairy

GnRH is used as the sole agent in a number of scenarios; often this is to assist the establishment of pregnancy in 'repeat breeder cows' (those that have been served 3 times yet failed to conceive).

1.) GnRH holding injection: A dose of GnRH is given on the same day as the cow is served. This will induce an LH surge and hence, ovulation. This program will benefit cows that have a delayed ovulation by more closely aligning insemination and ovulation.
2.) GnRH at 11-12 days: this is applicable for dairy cows only due to the difference in duration of follicular waves between dairy cows and other female bovines. 11-12 days after insemination a single dose of GnRH is given. This induces an LH surge causing the (ovulation and) luteinisation of the dominant follicle of the next follicular wave on the ovaries. This will remove the high levels of oestrogen production by these follicles, maximising the inhibition of oxytocin receptor expression on the endometrium, delaying the release of $PGF2_{\alpha}$. Ultimately this buys the embryo more time to signal its presence to the cow before luteolysis of the CL occurs, reducing early embryonic loss.

Ovsynch: this is one of the most well-known protocols for oestrus manipulation. It is only relevant to dairy cows as it is not suitable for bovines with three follicular waves. This program allows synchronisation of oestrus and ovulation and therefore removes the need for detection of heat (oestrus). It can also be used with insemination to observed oestrus following synchronisation. The timeline below illustrates the protocol;



The first GnRH that is given on day 0 of the protocol (not day 0 of the oestrus cycle) induces an LH surge causing ovulation of a dominant follicle with CL formation and the initiation of a new follicular wave. 7 days later $PGF2_{\alpha}$ is given to lyse the newly formed CL now it is sensitive to prostaglandins. Removing the CL and its progesterone secretion allows the next follicular wave's dominant follicle to finish maturing and in preparation for ovulation when the second GnRH dose is administered at day 9. Ovulation occurs 26-32 hours after the second GnRH hence, AI can then be performed at a fixed interval of 17-24 hours after the second GnRH administration (or to observed oestrus if preferred).

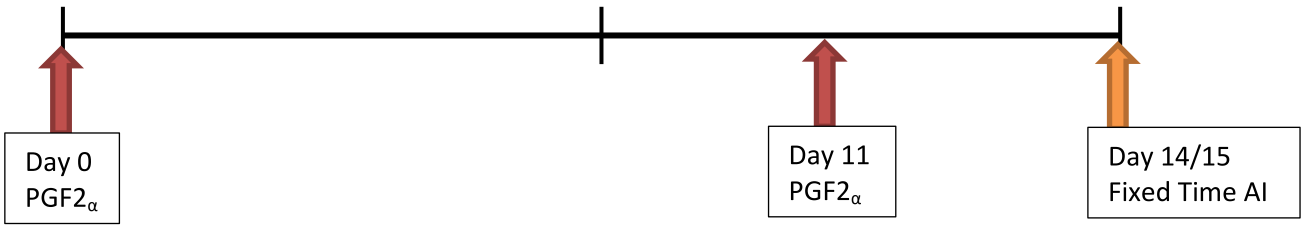
The response to the Ovsynch program is variable depending on the stage of follicular wave the cow is in at the time of the first induction of ovulation (i.e. when the program is started on day 0). It is reported that this program is more successful (greater conception rates) if the first GnRH induces ovulation of a dominant follicle. If the follicles on the ovary are not yet sufficiently mature then there will be no ovulation in response to this first GnRH. The role of the day 7 $PGF2_{\alpha}$ dose then becomes the luteolysis of a CL present on the ovary from a spontaneous ovulation that occurred in the few days before the protocol was started. Consequently, to increase conception rates achieved with Ovsynch, modifications to the traditional protocol have been made which allow pre-synchronisation, increasing the likelihood that cows will have a dominant follicle on the ovary at the time of the first Ovsynch GnRH dose. An example, 'Presynch-Ovsynch', is given below;

Through Pre-synch we can increase the likelihood that there will be a dominant follicle to be ovulated at the start of Ovsynch (day 0). This allows us to improve the subsequent conception rate from the Ovsynch protocol. Lysing any CLs present on the ovaries with $PGF2_{\alpha}$ administration before day zero will remove the progesterone-mediated suppression of spontaneous ovulation. After ovulation, the next follicular wave is initiated which enables us to reliably predict when there will be a dominant follicle capable of ovulation based on the well documented patterns of follicular waves in dairy cows. Two doses of $PGF2_{\alpha}$ are required for Pre-synch as if the first is given whilst there is no CL or an immature CL (less than 5 days old) on the ovary then the first $PGF2_{\alpha}$ dose will have no effect. However, we can be certain that 14 days later there will be a receptive CL regardless of if luteolysis occurred at the first dose or not. Hence, by the start of the Ovsynch program the next follicular wave will have occurred producing a dominant follicle ready for induction of ovulation.

<u>Manipulation Programs: Sucklers</u>

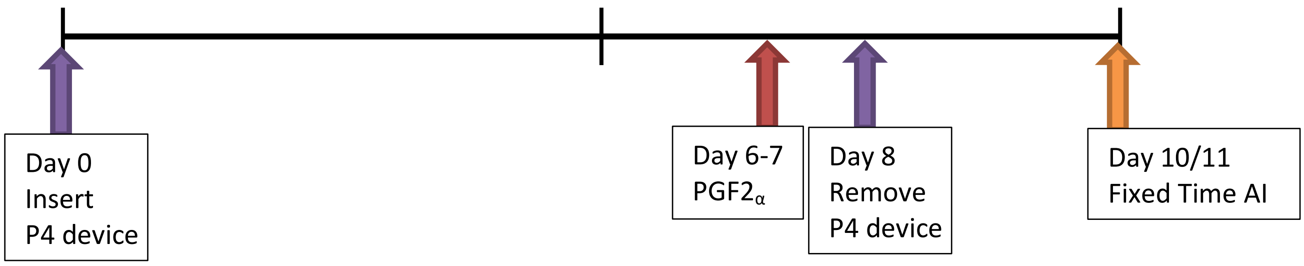
Whilst oestrus manipulation is more widely used in the dairy sector than the suckler herd sector there are still numerous uses for such intervention. These include but are not limited to; synchronisation of heifers to optimise calving timing, facilitating a tight calving interval, for the use of AI (especially fixed time AI) and for breeding technologies such as Embryo Transfer. Beef synchronisation programs can be broadly categorised as 'prostaglandin based' and 'progesterone-device based'.

An example of a prostaglandin-based protocol is illustrated below:



Fixed time AI is used at 84 hours after the second prostaglandin dose on day 11. Some reproductive technicians will instead opt to perform double insemination at 72 and 96 hours in an attempt to improve conception rates following this protocol.

An example of a progesterone device based protocol is illustrated below:



A progesterone (P4) device such as a CIDR or a PRID is inserted into the vagina of the cow/heifer on day 0 which will mimic the physiological effects of a CL. 24-48 hours before device removal, a dose of $PGF2_{\alpha}$ is administered to induce the luteolysis of any CLs that are naturally present. On day 8 after insertion the progesterone device is removed from the vagina (in effect mimicking the lysis of the artificial CL). The removal of all progesterone suppression (natural and iatrogenic) of GnRH release will permit an LH surge to occur and ovulation of a mature dominant follicle. Fixed time AI is used at 56 hours after the progesterone device is removed (or double insemination can be used at 48 hours and 72 hours post-removal).