Hormone use for the synchrony of oestrus

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Introduction
Understanding hormone function, and the timelines for hormone action, can assist with devising treatment regimens for manipulating the bovine oestrous cycle. The development of synchrony regimens can be further enhanced when an understanding of hormone function is coupled with critical physiological timelines such as sperm longevity within the female reproductive tract and post-ovulation oocyte viability. The aim of this presentation is to identify some key physiological events relevant to fertilisation, then provide a brief overview of reproductive hormone function, with specific reference to what is known regarding the timelines associated with their action. The hormones of specific interest include, GnRH, prostaglandin, progesterone, and oestrogen.

Significant events influencing insemination timing
The overriding assumption with inseminating cattle is that the best time to breed is as close to, but before, ovulation. While there does not appear to be a definitive study to confirm this assumption, the body of evidence is substantial. This evidence is based around the timing and action of the LH surge, the longevity of the secondary oocyte post-ovulation, the duration of sperm capacitation within the female tract, and the longevity of the sperm reservoir within the female tract.

The LH surge is important for inducing ovulation and stimulating maturation of the oocyte. It stimulates the oocyte to wake up out of its meiotic prophase stasis and continue on with the process of meiosis so that it can become mature (secondary oocyte) and be able to be fertilised. Most studies suggest the period from the commencement of the LH peak to ovulation is around 27 to 29 hours and is fairly consistent.

Once the oocyte has undergone maturation (at the time of ovulation) it has a very clearly defined viable duration. While there’s some conjecture as to the length of oocyte viability, it is somewhere in the range of 6 to 10 hours maximum that the oocyte will remain viable after ovulation. From a practical viewpoint, six hours is about the maximum time for post-ovulation insemination before oocyte viability becomes questionable.

Another important timeline is the duration taken for sperm to undergo the capacitation process, which is essential prior to fertilisation being possible. Capacitation is sometimes referred to as “Destabilisation of the sperm head membrane”, referring to the stripping out of cholesterol and proteins, increased fluidity, membrane labilisation, increased calcium permeability, increased cAMP and increased motility known as hyperactivation. Capacitated sperm do not survive long once the process is complete. Capacitation takes 4 to 6 hours to complete. There is suggestion that this process is shortened in frozen-thawed semen.

There is also the need to consider sperm longevity within the female reproductive tract. Over the aeons, survival mechanisms have been put in place to give the best shot of fertilisation occurring. So rather than all sperm undergoing capacitation, reaching maturity and all dying at once, some sperm remain quiescent for a period, providing a rolling wave of those processes occurring. This “staged” capacitation means that there is a huge
reservoir effect, allowing leeway with regard to when fertilisation can occur.

Importantly, there is a need to differentiate between the types of semen being inseminated including the differences between fresh semen, frozen-thawed semen and in more recent times frozen-thawed and sex sorted semen. Eg fresh semen longevity is approximately 48 hours. Frozen-thawed semen longevity is approximately 12 to 24 hours. With these timelines in mind, the following hormonal events can be considered.

**Oestrous Cycle Overview**
An overview of the bovine oestrous cycle is presented in Figure 1.

![Figure 1. Morphological and endocrinological events in the oestrous cycle of the cow](image)

**Figure 2.** Follicular waves comprise a cohort of follicles - often up to 20 or more. Follicle deviation is the term used when one follicle achieves steroidogenic capability and hormonal dominance over the remainder of the cohort. This occurs when the follicles reach approximately 8 mm. At this point subordinate follicles undergo atresia due to a lack of FSH support (in part due to inhibin from the dominant follicle). If the dominant follicle emerges at a time of progesterone dominance, it will eventually become atretic and a new follicular wave will emerge. If the dominant follicle emerges when progesterone is low (or if an exogenous source of GnRH or hCG is administered), it can proceed to ovulation.

**The Role of Progesterone and Oestrogen**
An overview of the role of progesterone and oestrogen is shown in Figure 3.
Figure 3. This image demonstrates the dual role of oestradiol during the bovine oestrous cycle. The left-hand side represents the hormonal activity during progesterone (P4) dominance, while the right-hand side represents hormonal activity in the absence of progesterone.

In the presence of P4 dominance (luteal phase), GnRH pulses are mainly low frequency and high amplitude - conducive to FSH release and follicle development (recruitment). Only after P4 concentrations reach baseline (right-hand side) can high frequency, low amplitude GnRH pulses, (that are stimulated by a rise in oestradiol), generate an LH surge. The inhibitory effect of P4 on the hypothalamic/pituitary axis can be overridden by the administration of exogenous GnRH. Therefore, midcycle, dominant follicles can be induced to ovulate (or at least luteinise) by the administration of exogenous GnRH. Importantly, any residual P4 at the time of insemination (eg resulting from failure of the CL to be completely destroyed subsequent to a synchrony program), may interfere with LH pulse frequency and ovulation.

Other important roles of P4 which need to be considered when designing synchrony programs include:
- The progesterone priming effect on the hypothalamus which enhances oestrus behaviour during the subsequent oestrogen dominant period.
- Newly emerging evidence that the presence of P4 prior to the E2 stimulus seems to enhance the E2 effect – possibly due to increased E2α receptors on hypothalamic kisspeptin neurons.

These points need to be considered when designing synchrony programs. Minimising the exposure of cows to progesterone in the lead up to the induced ovulation may reduce oestrus behaviour and ovulatory response.

Oestrogen in combination with progesterone provides extreme suppression of gonadotrophin support both at the hypothalamic and pituitary level (Figure 3). This suppresses all follicular wave progression when utilised in synchrony programs, results in the emergence of a new follicular wave in approximately 3 days after the commencement of treatment. This means that the majority of candidates (close to 100%) will have a new follicle emergence as a result of P4/E2 treatment, the dominant follicle resulting from which, will be only about 7 days of age at the completion of treatment in a traditional 10-
day CIDR program, and approximately 9 days of age at the time of insemination.

Importantly, all oestrogens (E2) aren’t created equal and care needs to be taken when utilising E2 conjugates in synchrony programs. Oestradiol and its esters have distinct durations of action:

Oestradiol 17B is one of the native oestrogens and has a relatively short half-life (~2.5 hours), with a duration of action from a single administration suggested at approximately 15 hours.

Oestradiol valerate is still used in some synchrony products eg synchromate -B/crestar and has an estimated duration of action ranging from 14 to 21 days.

Oestradiol benzoate (ODB), which is commonly used in synchrony programs, has an estimated half-life of approximately 12 hours, with a duration of action from a single administration of in the range of 2 to 3 days.

If oestrogen is used to assist with the induction of ovulation, the duration from E2 administration to the LH surge is approximately 20 hours. When the duration from LH surge to ovulation is factored in (27 to 29 hours), it can be estimated that the duration from E2 administration to induced ovulation will be somewhere close to 48 hours. The timing of such an injection can be critical, because if P4 is still present when E2 is administered, there may be inadvertent interference with the LH surge.

The injection of oestradiol benzoate also results in premature luteolysis in more than 40% of cows. This is again due to the extreme suppression of the gonadotrophins (in this case the main interest is in LH) resulting from the combination of oestrogen and progesterone. The reduced LH means there is a lack of gonadotrophin support for any cows that happen to have a young corpus luteum at the time of administration. This will be around 25% to 30% of a randomly cycling group of animals.

Critical points:

- E2 in the presence of P4 will create a profound block of LH and FSH. This can be good for resetting the follicular wave in ALL treated animals, with the emergence of a dominant follicle approximately 3.5 to 4 days after treatment. It can also be good for inducing premature luteolysis in early dioestrous cows. However, it can be detrimental if there is residual P4 in the lead-up to ovulation.
- P4 prior to the administration of E2 enhances the oestrogenic effect. This includes improved expression of oestrus and improved preovulatory GnRH release from the “surge centre”.

The Role of GnRH

GnRH is released from the hypothalamus. Traditional understanding describes two distinct areas of hypothalamic control of GnRH release. The anterior hypothalamic nuclei are often referred to as the “surge centre” as they control the LH surge under the positive feedback of E2, in the absence of P4. The middle hypothalamic nuclei often referred to as the “pulse generator” control the tonic release of GnRH and subsequently the tonic release of FSH and LH. E2 and P4 combined have a profound suppressive effect on the anterior hypothalamic nuclei.

After exogenous GnRH administration, there is a rapid increase in LH pulse frequency leading to commencement of an LH surge within 60 minutes. 100ug GnRH induces a greater LH peak response, but of slightly shorter duration (6 hours cf 10 hours), than a natural endogenous surge. If a dominant follicle is present (even during dioestrous) normal ovulation (or at least luteinisation of the granulosa and theca interna cells), usually occurs
within 29 hours of GnRH administration. Either way (ovulation or luteinisation), new luteal tissue will be present that can respond to PGF$_{2\alpha}$ after 5 days of maturation. After ovulation, a new follicular wave can emerge and a new dominant follicle with ovulatory capacity will be present within 2.5 to 3 days after the ovulation (about 4.5 days after the GnRH injection). The functional lifespan of this new follicle is 8 to 11 days (12 to 15 days after GnRH injection).

Critical points:
- Injection of GnRH will only induce the emergence of a new follicular wave if it is administered in the presence of a dominant follicle. Otherwise, there will be no effect on follicular wave dynamics. In a randomly cycling group of females, there may only 40% of two follicular wave animals and perhaps 55% to 60% of three follicular wave animals, (so an overall of approximately 50% of animals) that will have their follicular wave reset using GnRH at the commencement of a program.
- Only after the decline in P4 can GnRH release generate high frequency pulses of LH, leading to a surge. This may be relevant to the timing of PGF$_{2\alpha}$ administration in synchrony programs and when AI is performed relative to progesterone device removal.
- Assuming there is a receptive follicle, there is approximately 29 hours between GnRH administration and ovulation. This is relevant to the timing of GnRH administration in relation to insemination.

The Role of Prostaglandins
There is a well-established understanding that the bovine CL needs to be greater than 5 days of age before it is reliably responsive to the luteolytic effects of PGF$_{2\alpha}$. Given that the day 18 CL will undergo natural luteolysis, this means there are approximately 12 days of the bovine cycle that will be responsive to PGF$_{2\alpha}$.

It is also well established that steroids regulate the pulsatile control of GnRH/LH secretion through the hypothalamic/pituitary axis. Steroid hormones also modify the LH responsiveness of the pituitary to GnRH injections that bypass GnRH regulation by the hypothalamus (see Figure 3). An important consequence of this in relation to developing synchrony regimens was highlighted by a study over 40 years ago, on the effect of prostaglandins on the lysis of the bovine CL (Thatcher and Chenault, 1976). In their study, plasma LH responses were evaluated following injections of GnRH (100 ug) given to dairy heifers (n=25) in the dioestrus/luteal phase of the cycle (Days 9 to 15) at 0, 12, 24, 46 or 60 h after a luteolytic injection of PGF$_{2\alpha}$. Maximum LH response was not reached until 48 and 60 h after PGF$_{2\alpha}$ injection. However, it was not until 60 h that there was no temporal increase in plasma progesterone indicative that functional and structural regression of the CL was not complete until 60h after injection of PGF$_{2\alpha}$. This has significant implications in reproductive management procedures.

Critical point:
- Provide plenty of time for complete luteolysis to occur after PGF$_{2\alpha}$ administration in synchrony programs. This will ensure that no endogenous P4 is present to interfere with the exogenous progestogen control of the cycle. At least 48 hours, and possibly up to 60 hours is needed.