EVALUATING THE BENEFIT OF eCG & hCG INCLUSION ON REPRODUCTIVE OUTCOMES FOLLOWING SYNCHRONISATION & FIXED TIME ARTIFICIAL INSEMINATION OF BEEF HEIFERS

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Project title
‘Evaluating the benefit of eCG & hCG inclusion on reproductive outcomes following synchronisation and fixed time artificial insemination of beef heifers.’

INTRODUCTION

Project background, context and need

Youngstock represent both the future of the beef suckler herd as well as a significant cost of production. EBLEX (2010) describe how the gap in business performance between the top third and sample averages has widened. The best performing enterprises have produced improved margins despite higher costs. Caldow et al (2007) proposed a five point plan to manage beef cow productivity. Point 1 is ‘heifer management’. All too often heifer replacement management remains an afterthought for the beef suckler herd, with inappropriate genetic selection for future suckler cow production and strategically poor integration to the overall farm business.

Using strategies to ensure that heifers calve before the main herd and at around 65% of mature weight are vital (Caldow et al, 2007). Synchronisation and fixed time artificial insemination (AI) of maiden heifers is an underutilised opportunity to achieve both the above targets as well as critically
to select appropriate bull genetics for positive calving ease and negative gestation length EBVs. Currently, heifers are frequently naturally mated inappropriately by terminal sire bulls, at best selected for cow mating, with poor outcomes for future herd breeding potential.

Artificial insemination (AI) represents an opportunity for improvement in the beef suckler herd. Bull genetics may be economically selected on EBVs targeted specifically to future breeding performance rather than inappropriate carcase traits associated with terminal sires. Use of breeding technologies represents an important opportunity for beef suckler herds to mitigate greenhouse gas emissions by improving productivity and efficiency (Chadwick et al 2007). Oestrus synchronisation protocols exist for fixed time AI (Penny 2005) but results can be disappointing when compared to natural service. Therefore, this study aims to investigate opportunities to improve reproductive outcomes using modifications of existing programmes.

Progesterone (P4) treatment regimes are commonly used in AI of beef heifers, but equine Chorionic Gonadotrophin (eCG) administration at P4 removal stimulates both dominant follicle growth and oestradiol production. Human Chorionic Gonadotrophin (hCG) administration at insemination may improve corpus luteum (CL) quality and subsequent progesterone production with reduced losses from subsequent early embryonic death (Santos et al 2001). This pilot study investigates the effect of eCG and hCG on performance of AI programmes in beef heifers as measured by blood progesterone assay and related to subsequent reproductive outcomes i.e. pregnancy success.

**Synchronisation programmes in beef suckler systems**

In order to obtain maximum benefit from using AI, synchronisation can be used to further tighten the calving pattern, avoid the need for constant observation for heat and to improve the timing of AI.

Before the limitations and merits of differing synchronisation protocols can be discussed a brief understanding of the normal reproductive cycle of the cow is required.
The length of the oestrus cycle is controlled through progesterone production by the corpus luteum (CL), this progesterone blocks production of gonadotrophin releasing hormone (GNRH) in the hypothalamus, which in turn reduces luteinising hormone (LH) and follicle stimulating hormone (FSH) production in the pituitary.

Under conditions of low LH and FSH levels dominant follicles will not fully mature or ovulate.

At around day 17-18 of the cow's cycle the uterus produces prostaglandin (PG). This causes regression of the CL and a rapid fall in blood progesterone levels.

This removes the inhibition of GNRH production which in turn leads to rising FSH and LH levels allowing a dominant follicle to mature. The maturing follicle releases increasing amounts of oestrogen which acts on the pituitary gland making it produce even more FSH and LH in a positive feedback cycle. Oestrogen and LH levels increase very quickly; this is called the 'LH surge' and results in ovulation and oestrus.

Once the egg has been released the ruptured follicle forms a new corpus luteum and the cycle begins again.

Fig. 1 Normal oestrus cycle
In controlled breeding programmes synthetic hormones are used to control the breeding cycle of the cow. The following hormones are used in different ways during different programmes.

**GnRH – (Gonadotrophin Releasing Hormone) eg Receptal™**: This hormone acts on the pituitary gland to stimulate release of FSH and LH. This is used to cause three main effects:

1. Ovulation of dominant follicles leading to the formation of a corpus luteum which may be available for luteolysis using prostaglandin.
2. The stimulation of further follicular development through ‘new wave emergence’.
3. Ovulation of mature follicles to control the time at which the oocyte (egg) is released and therefore optimise timing of insemination.

**PG – (Prostaglandin) eg Estrumate™**: This chemical signal acts on the corpus luteum causing it to regress. The corpus luteum produces progesterone and so its destruction causes a sudden drop in the level of progesterone; this removes the inhibition of follicle development and allows a new dominant follicle to mature ready for ovulation in 2-5 days. If there is no CL present then the PG will have no effect.

**CIDR or PRID (Intra vaginal Progesterone Releasing Devices)**: These vaginal implants supply progesterone to the female and so influence the feedback mechanisms of the hypothalamo-pituitary-gonadal axis as described above. In effect, it inhibits follicle development and ovulation.

**hCG - (Human Chorionic Gonadotrophin)**: Delivers a LH-like action to promote ovulation and luteinisation (formation of the CL).

**eCG - (Equine Chorionic Gonadotrophin)**: Delivers FSH-like action to stimulate follicle recruitment, growth and maturation.

There are several methods of oestrus synchronisation available, each carrying benefits and drawbacks. Most originate in the dairy sector and this has implications for their application and suitability to the beef sector.

**Double PG** uses two prostaglandin injections administered 10-12 days apart, the first PG should cause luteolysis of the corpus luteum present in any cattle beyond day 8 in the ovarian cycle. Cattle will then be returned to the start of the cycle and as such 10-12 days later should have a functioning CL and be receptive to prostaglandin. The limitations of this programme are that it results in a relatively wide deviation in time of ovulation from one cow to another and as such double A.I. is recommended at 72 and 96 hours following the second PG. Alternatively the cow can be served once
at 84 hours although this can be associated with lower conception rates. The regime is not appropriate if a cow is not cycling normally.

**Ovsynch** was originally developed in the USA and has been used successfully for many years primarily in dairy cattle. It represents the blueprint upon which most of the other programs are built. The first GnRH injection causes ovulation of any dominant follicles leading to CL formation. The PG at 7 days then causes regression of this CL allowing a new dominant follicle to mature before the second GnRH injection 56 hours later causes ovulation ready for fixed time AI.

**Select-synch** uses the same protocol as Ovsynch but only cows that are actually observed in oestrus are served. This programme may improve conception rates. However, cows that have ovulated may not be served with a consequent reduction in submission rates, as they may not exhibit oestrogen driven behavioural signs of heat.

**Co-Synch** uses a similar protocol to Ovsynch with the exception that the second injection of GnRH is postponed until 72 hours after PG and given at the same time as the cow is served. The benefit of this with regard to beef cattle is that it reduces the number of times that the cattle require handling, thus reducing stress for the cattle as well as reducing labour input.

**CIDR-synch** uses a CIDR to supplement the levels of natural progesterone produced by the corpus luteum. The CIDR is inserted on day one at the same time as the initial GnRH injection and is left in place for up to 12 days and preceded 24 or preferably 48 hours before withdrawal with an injection of PG. Progesterone supplementation for longer than 12 days may lead to reduced oocyte viability as a consequence of prolonged follicular dominance.

**Modified Ovsynch/CIDR-synch** uses eCG instead of the first GnRH injection, with FSH action for stimulation of follicular growth and if used in conjunction with intra vaginal progesterone has application in both growing beef heifers and suckler cows synchronised relatively soon post-calving in poor body condition, that calve later in the calving season.

A further modification to the programme uses hCG to promote luteinisation and formation of accessory CL to increase progesterone levels post-insemination and reduce embryonic death.
Fig. 2 Synchronisation programmes
**Triple-synch** is a system devised by the Scottish Agricultural College (SAC) which aims is to reduce the need for sweeper bulls by synchronising returns to oestrus by cows that have failed to conceive. Allowing second and third rounds of AI as well as recruiting any late calvers into subsequent rounds of AI.

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<td>Inject PG</td>
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<td>9</td>
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<td>35</td>
<td>AI (am) LATE CALVERS and any other returns to heat</td>
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<td>50</td>
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<td>53</td>
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<td>Careful observation for heat and serve as required</td>
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<tr>
<td>58</td>
<td>AI (am) any cows seen bulling and all non-pregnant group.</td>
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Table 1 Triple synch programme

The advantage of this programme is that it gives three opportunities of cows to be served with fixed time AI without the need for a sweeper bull. Disadvantages include labour input and cost. Care must be taken to ensure CIDRs are thoroughly cleaned, disinfected and appropriately stored prior to re-insertion and the modification to a lower progesterone dose in CIDR since first devised may require fresh CIDR inserts at each stage of the programme.

**HYPOTHESIS:**

‘Maiden beef heifers receiving eCG & hCG as part of AI synchronisation programmes are more likely to achieve pregnancy and successful reproductive outcomes’
Pilot study Method

Herd background

Maiden heifers were recruited from a beef herd of approximately 200 cows to reduce potentially confounding inter-herd factors. The herd comprises spring and autumn calving herds of approximately 130 and 70 commercial crossed suckler cows, respectively, naturally bred to terminal sires including Charolais, Simmental, Limousin and Black Limousin. Although previously the breeding herd was primarily dairy Friesian crossed with Aberdeen Angus or other native beef breeds, over time the Holstein influence had prompted a change in policy and replacements are now homebred with increasing inclusion of terminal sire beef breeds and in particular Limousin.

The enterprise is a mixed farm with grazing of permanent water meadow pasture complemented by a substantial arable business benefitting the livestock aspect with both straw and cereals such as barley and wheat to feed. A mixer wagon offers the flexibility of feeding a total mixed ration (TMR), but a range of feeding strategies are utilised with entire beef bulls still reared on a conventional ad lib cereal hopper system. Cows and fattening steers and heifers are fed a TMR based around a mix of clamp grass silage, straw and cereals with a purchased protein balancer. Beef is sold deadweight through ABP and carcase conformation classification consistently achieves E & U.

Breeding females graze permanent pasture during the summer and this has been significantly affected by flooding of low-lying water meadows in recent years, together with the emergence of liver fluke as a problem in the adult and youngstock herd in this time. During the winter, breeding cows and replacement heifers are fed on a TMR based mainly on clamp grass silage with predominantly straw for spring calving cows but autumn calving cows and growing heifers are supplemented with small amounts of cereals and protein blend during times of high demand such as early lactation and adjusted by body condition score.

Herd health status has improved over recent years and although not formally part of a CHeCS cattle health scheme, annual heifer cohort screening has showed no evidence of circulating BVD or leptospirosis for two years and the herd is TB free with no evidence of Johnes disease on cull cow screening tests. IBR has been a significant issue and a vaccination programme with live intranasal vaccine in youngstock at housing is supported by use of intranasal vaccine against RSV and Pi3 viruses in spring born calves following previous IBR and RSV outbreaks. Building and ventilation modification has been implemented in the last two years as part of a ‘pneumonia MOT’ and respiratory health has improved subsequently.
Breeding management

The herd uses mainly natural service with a range of bull breeds including Charolais and Simmental but mainly Limousin and Black Limousin. Over a number of years, as fewer replacements have been purchased, heifers have been served by artificial insemination (AI) at the very start of the breeding season before follow-up with natural service. However, disappointing pregnancy results had challenged this policy.

Study recruitment

A sample size of approximately 30 heifers described the available pool of replacements on this farm. Heifers were of homebred mainly Limousin cross type in variable body condition but average BCS was 3.75 at service. The average weight of the heifers was 457kg, with most study animals having a date of birth in March/April 2011 (See appendix 1 for complete heifer age and weight data).

Figure a & b: Photo of heifers on day O – first day of AI

Figure a:
Study method

The recruited heifers were gathered and examined in a race and crush system prior to insertion of an intra vaginal progesterone releasing device ‘P4’ (CIDR; Pfizer Animal Health) and progesterone levels were measured in blood samples using ‘Ridgeway Target’ kits, permitting a numeric assay result to be recorded by a breeding technician at Bishopton Breeding reproductive laboratory. All breeding heifers were sampled at the start of the breeding programme and subsequently as below:

(i) time of progesterone (P4) device insertion (day –9)
(ii) time of prostaglandin F2 alpha (PG) administration (day-3)
(iii) day 0 (oestrus/ insemination)

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Table 2 Programme and sampling protocol

Body condition score at P4 insertion was recorded.
The recruited heifers were randomly assigned to either study or control groups and administration of 400iu of eCG (PMSG; MSD) to study animals occurred at P4 device removal (day -2), with GnRH (Receptal, MSD) injection given to control animals on the same day. Administration of 1500iu hCG (Chorulon, MSD) also occurred on day 0 at fixed time artificial insemination with GnRH injection administered to control animals. A double fixed time insemination technique was used with AI technician attending on two consecutive days and heifers gathered and inseminated in the race and crush system. Two different breeds of AI sires were used on the two days to evaluate success of timing in the programme.

ALL heifers entered the synchronisation programme and therefore all received both a P4 insert with prostaglandin before removal. The study animals received eCG and hCG whereas the control animals received two injections of GnRH:

- **CONTROL** heifers receive two doses of GnRH, one at P4 removal (day -2) and one at insemination (day 0) - (RR)
- **STUDY** heifers receive a dose of eCG at P4 removal (day -2) and hCG at time of insemination (day 0) - (HE)

Sweeper bulls were introduced to the heifer group 10 days post second insemination. Pregnancy diagnosis (PD) was performed at approximately 50 days after insemination (day 0) by ultrasound and repeated 2 months later. This allowed determination of pregnancies achieved through the AI programme in contrast to those by natural service through accurate pregnancy dating using early PD. The heifers were managed as one single group at grazing during the entire study period.

**RESULTS**

Table 3 below shows the variation in progesterone levels at CIDR insertion, PG injection and insemination together with pregnancy diagnosis result at early ultrasound stage and comparison with study and control groups:
Of the 30 females synchronised in this study, table 3 (above) shows how only 5 (1 in 6) were PD positive at the initial ultrasound examination at a stage of pregnancy consistent with the AI programme. A further two animals were PD positive but at a stage consistent with the first cycle with the bull and natural service. All 5 of these PD positive animals had been under the modified study programme (HE) - no controls had become pregnant to AI in the control group (RR).

Secondly, variation in progesterone levels was apparent at the stages of sampling. This was expected at the initial sample, when females would be at any stage of the cycle, but test 2 (time of PG administration) was expected to show more animals at high progesterone and test 3 (time of insemination) more animals at low progesterone than was apparent.
DISCUSSION

This pilot study is based on a small sample size and consequently should be interpreted with care as the number of study animals is insufficient to deliver statistical significance. Reproductive success is hugely multifactorial and a number of issues are likely to have influenced the outcomes of this study in regard to pregnancy generation.

In order to maximise conception rate when using either natural service or AI careful management of cows and heifers during the weeks leading up to service is essential. Nutrition should be consistent and the animals should be kept in stable social groups for at least six weeks prior to service. Unfortunately, the summer of 2012 saw the greatest rainfall since records began and this is likely to have had a detrimental effect on nutrition as heifers were managed at grazing during this programme and so were exposed to massive variation in dry matter intake depending on weather conditions. Poor sunshine levels are also likely to have specifically compromised sugar levels in grazed grass with consequences for glucose metabolism and reproductive physiology.

The poor weather is also likely to have had a negative impact at the time of insemination as heifers were gathered in an outside race/crush system to serve and endured a torrential rainstorm during insemination which is likely to have compromised both semen handling with cold winds and stressed heifers standing in exposed, wet conditions. Semen quality when reviewed by batch post-pregnancy diagnosis was of moderate quality and could have contributed to disappointing conception rates, in addition to the above environmental factors.

The relationships between heat detection, AI conditions and embryonic mortality were described by Freret (2006) & Ponsart et al. (2008). Behavioural signs of oestrus used by the farmer to call the AI technician were related to early embryonic mortality (EEM). The frequency of EEM was significantly increased when signs of oestrus other than standing heat or mounting activity (alone or in association) were used to call the AI technician (Freret, 2006; Humblot, 2001; Michel, 2003).
Factors potentially contributing to reproductive outcomes

A number of factors were not modelled in this study. The following may influence reproductive outcomes:

**Infectious disease and embryonic mortality**

Reproductive outcomes may be influenced by infectious disease status; this study did not primarily investigate this issue. Bovine herpes virus -1 (BoHV-1) was endemic in the herd and bovine viral diarrhoea (BVD) had been an issue historically. It could therefore be speculated that infectious disease may have contributed to the poor reproductive performance of the herd (Nettleton, 2008; Booth and Brownlie, 2012).

Furthermore, during the summer of 2012, Schmallenberg virus (SBV) tracked northwards throughout much of England following incursion into southern regions of England during 2011. Although at this early stage in our understanding of the epidemiology and impacts of SBV it is impossible to make conclusive statements, it is quite possible that SBV had a negative impact on reproductive outcomes in this herd during this study. Along with many other herds in the region of the study, SBV seroconversion was noted during late summer of 2012.

**Semen quality**

Accurate identification of oestrus will not result in positive PD if semen quality, arising from either production, transport, storage or handling, is poor. Semen quality was not measured in this study prior to insemination, but was investigated following PD results. Dejarnette et al. (2004) described the issues regarding semen quality and sustaining the fertility of artificially inseminated cattle.

**Insemination technique**

McCoy et al. (2006) described the variation in performance between different inseminators, in particular between a technician service and ‘DIY’ AI inseminations by farmers. The current study did not address individual AI technique issues, which may have been significant in predicting reproductive outcomes. Contrary to planning, different AI technicians performed the inseminations on each day; the primary technician was unexpectedly delayed for the first insemination session.

**Genetics & embryonic mortality**

The impact of genetic selection for traits other than fertility was not considered in this study. Negative genetic correlations exist between milk production traits and fertility variables in dairy
breeds but a greater proportion of terminal sire beef breeds may have been significant in the genetic composition of these heifer replacements (Burns and others, 2011; Coffey and others, 2007).

Despite the above issues, it is interesting to note that it was only the ‘modified synch’ females (5/15) that achieved pregnancy and 0 control animals out of 15 became pregnant to AI. It would be of great interest to explore a larger scale study with further standardisation of conditions to explore the effect of programme modification to a statistically significant level.

**Summary - Factors to consider**

Artificial Insemination (AI) can bring a multitude of benefits to the suckler herd including access to more proven, better quality sires. It brings the opportunity to target sire selection for different groups of females; for example one bull for the heifers, a second bull to produce heifer replacements and a third for producing highest quality beef carcase calves. It can be used to tighten up the calving pattern leading to more uniform calves that are easier to manage and it can potentially reduce the requirement for keeping as many (if any) bulls.

**Factors to consider**

A successful synchronisation programme requires attention to detail in the management of not only cattle but also handling facilities and labour requirements and might therefore not be suited for every beef enterprise.

**Cattle**

- Feed a consistent ration on a rising plane of nutrition which meets all requirements including minerals
- Fit not fat – correct body condition score (BCS- around 2.5, exclude cows <2 or >4)
- Heifers need to be approximately 65% of their mature body weight at first service
- Free from disease
- Observed cycling before synchronisation or AI *
- Cows should have calved at least 60 days before the synchronisation programme begins*
- Pre-service vet checked

* Progesterone based synchronisation protocols can help overcome anoestrus and be used in cows calved <60 days
Facilities

Facilities need to be adequate to easily handle several interventions during the synchronisation and AI process, and then a high proportion of cows calving over a short period of time.

- Are the cattle housed or at pasture?
- The majority of synchronised females will calve within a 2 week period and a significant proportion may calve on one day

Labour

- Sufficient to cover concentrated requirements at breeding and calving
- Programmes can range from handling cattle only once to handling over 5 times in three weeks
- Experienced, skilled and disciplined labour is important to reduce stress and time
- DIY AI may make some synchronisation programmes more workable, remembering that there will pressure on an inseminator who is not doing this number routinely

AI technique and semen quality

- Good technique essential
- Screening semen quality advisable
- Semen handling facilities and technique critical

Cost Benefit

- Medicine costs can vary depending the synchronisation programme, ranging from below £8/cow to over £18/cow
- It is important to equate these costs per calf born against costs of bulls (eg approximately £29/calf for 2x AI versus £45/calf for bull maintenance)
- It is important to weigh these up with the predicted conception rates and subsequent impact on costs such as labour later on in the production cycle.
- Consider any costs after the synchronisation programme – eg sweeper bull
- Consider the risks associated with bull purchase, both from disease, and from sub-fertility
### Appendix 1: Study animal details (15/06/2012)

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<td>0.55</td>
<td>10/04/2011</td>
<td>B.A</td>
</tr>
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<td>186</td>
<td>441</td>
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<td>B/LIM</td>
</tr>
<tr>
<td>154 C</td>
<td>658</td>
<td>-</td>
<td>19/05/2001</td>
<td>LIM</td>
</tr>
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<tr>
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<td><strong>457</strong></td>
<td><strong>0.62</strong></td>
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</table>

**B/LIM** - Black Limousin  
**B.A** - Blonde D’Aquitaine  
**C = Cow**

Weight recorded at day of insemination and daily liveweight gain (DLWG) measured from weight at weaning to insemination day.
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References

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