

# Pregnancy rate after fixed-time artificial insemination of suckled beef cows subjected to a cosynch protocol with either buserelin or hCG as ovulation inducing agent



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## ABSTRACT

The aim of this study was to document the effectiveness of the implementation of a cosynch protocol and fixed time artificial insemination in suckled beef cows kept under range conditions in Germany. In addition, the suitability of hCG administration as an alternative to the GnRH analog buserelin (Receptal®, Intervet, Germany) as ovulation inducing agent was investigated. The 120 Simmental and 120 German Angus cows were randomly divided into four groups. Cows of Group 1 were subjected to the classical cosynch protocol, constituting administration of GnRH, followed, 7 days later, by prostaglandin F<sub>2α</sub> and, after 2 days, another GnRH administration and simultaneous AI. In cows of Group 2, hCG was administered instead of GnRH at the beginning of the protocol, in Group 3 at the end and in Group 4 at the beginning and at the end. Inseminations with frozen-thawed semen from Limousin, Charolais, Simmental and German Angus sires were carried out by the same skilled inseminator. Blood samples collected 11 days before and on days 0, 7 and 9 of the cosynch protocol were analyzed for progesterone concentration. The study extended over three consecutive years. Of the total of 489 inseminations, 53% resulted in pregnancies (Simmental: 60%; German Angus: 48%). When GnRH was administered at the beginning and at the end of the cosynch protocol (Group 1), pregnancy rate across breeds was 65% (Simmental: 61%; German Angus: 68%); likewise when hCG was substituted for GnRH at the end of the protocol (Group 2). Pregnancy rate across breeds reached 48% when hCG was given at the beginning (Group 3) and was lowest (37%) when hCG was given at the beginning and at the end. German Angus cows responded particularly poorly to hCG treatment. In cows that were cycling, as indicated by serum progesterone levels 11 and 0 days before treatment, pregnancy rate across breeds and treatments was highest (57%) if the cosynch protocol was initiated at a stage of the cycle when peripheral progesterone levels were on the rise. In non-cycling cows, representing only 13% of the herd, contrary to expectation pregnancy rate reached 65%. In conclusion, implementation of fixed-time artificial insemination in suckled beef cows kept under local range conditions led to satisfactory pregnancy results, especially if the cosynch protocol was initiated at a stage when progesterone levels were on the rise. Substitution of hCG for GnRH proved to be of no advantage; in fact, when administered at the beginning of the protocol it resulted in a detrimental effect on fertility.

## 1. Introduction

With increasing farmland falling fallow and high producing dairy cows for the most part having little or no access to pasture, beef cattle ranching is expanding in Germany and other European countries. Under extensive husbandry conditions artificial insemination is difficult to conduct unless estrus synchronization is implemented. While fixed-time

artificial insemination has become fairly common in the dairy industry, its potential in beef cattle in much of Europe is limited (Colazo and Mapletoft, 2014) and has not been fully documented.

Various studies (Geary et al., 1998, 2001a, 2001b; Vasconcelos et al., 1999; Moreira et al., 2000a, 2000b; Cartmill et al., 2001; Keith et al., 2005) suggest that reproductive performance in cows may be impaired by the implementation of ovsynch or related synchronization

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protocols, presumably due to failure to induce ovulation at the initial GnRH treatment, lack of response to prostaglandin  $F_{2\alpha}$  administration and increased incidence of corpus luteum insufficiency. The latter is frequently associated with the formation of functionally inferior corpora lutea, conceivably ascribable to a sub-optimal LH surge induced by administration of GnRH. There are two options to minimize this problem (a) replace GnRH analogs by human Chorionic Gonadotropin (hCG), extracted from the urine of pregnant women, that efficiently binds to LH receptors (De Rensis et al., 2010) and is known to have a long half-life (Schmitt et al. 1996b). Hence, it was anticipated that hCG may better support ovulation and early corpus luteum development than the sub-optimal LH surge following ovulation induction with a GnRH analog, hence, generating corpora lutea of higher functionality (Schmitt et al. 1996b) and (b) use GnRH analogs instead of natural GnRH in the synchronization protocol, as such analogs are more potent and have longer half-life than natural GnRH (Chenault et al., 1990).

The aim of the present study was to (a) assess reproductive performance following a fixed-time artificial insemination protocol in suckled cows of two of the most common German beef breeds and (b) attempt to improve the pregnancy rate by substituting hCG for a GnRH analog at the beginning, at the end and at beginning and end of the cosynch protocol.

## 2. Material and methods

The experiment was conducted during the breeding seasons of three consecutive years at the experimental farm Rudlos of Giessen University (50°36'N, 9°25'E, 400 m NN) on a herd of 240 pluriparous beef cows consisting of equal numbers of two of the three most common German beef breeds, the meat-type Simmental and the German Angus breed. Simmental is the prevailing breed in much of Southern Germany, Austria and Switzerland; German Angus was originally derived by crossing Aberdeen Angus with various dual-purpose breeds. The cows of the herd were randomly assigned to four groups, taking breed, parity and post-partum-interval into consideration (Fig. 1). Cows of Group 1 were subjected to a classic cosynch protocol, constituting an initial GnRH administration, followed, 7 days later by prostaglandin  $F_{2\alpha}$  and, after 2 days, another GnRH administration and simultaneous AI. In cows of Group 2, GnRH was replaced by hCG at the beginning of the protocol; in cows of Group 3 at the end; and in Group 4 GnRH was replaced by hCG at the beginning and at the end (Fig. 1). Treatment commenced between 39 and 140 (mean 70) days post-partum. The agents administered were GnRH analog buserelin (2.5 mL Receptal®, Intervet Germany), human chorionic gonadotropin (1500 IU Chorulon®, Intervet France) and the prostaglandin  $F_{2\alpha}$  analog cloprostenol (2 mL Cyclicx®, Intervet, Germany).

During the years 2006, 2007 and 2008, 237, 158 and 143 cows, respectively, were treated. Each year cows were treated as separate entities randomly allocated to treatment groups, regardless of cyclicity, stage of the estrous cycle or previous treatment. They were inseminated with frozen-thawed semen from 5 Limousin and 5 Charolais sires, randomly distributed, to produce F1 crosses, and from 5 German Angus

and 5 Simmental sires to produce purebred offspring. Overall there were 212 inseminations with Limousin, 203 with Charolais, 41 with Simmental and 33 with German Angus semen. The cows of all treatment groups were inseminated by the same experienced inseminator early in the pasture season between April and June. The inseminator was instructed to rate estrous symptoms (vulvar turgor, color of vestibule, amount and viscosity of vaginal mucus, uterine tone and patency of the cervix) on a scale of 0–2 (0 = no evidence, 1 = weak evidence, 2 = strong evidence). The ratings added up to a maximum of 10, such that the score describing intensity of estrous symptoms ranged between 0 and 10. Three weeks after insemination bulls were introduced to the herd for 90 days to “clean up” return cows.

Blood samples were collected via coccygeal venipuncture 11 days before treatment (Day – 11), at the onset of treatment (Day 0), at prostaglandin administration (Day 7), on the day of ovulation induction and insemination (Day 9) and 33 days after insemination (Day 42) (Fig. 1). Blood was centrifuged at  $1600 \times g$  for 10 min and serum was stored at  $-20^\circ\text{C}$ . In the first four samples of each cow progesterone concentration was measured by ELISA according to Van de Wiel and Koops (1986) modified by Moeller (1991); the last sample was analyzed for content of pregnancy associated glycoprotein (PAG) (Friedrich and Holtz, 2010). Intra- and interassay-coefficients of variation for the progesterone assay were 8.8% and 11.0% (Moeller, 1991); for the PAG assay, 13.2% and 16.3% (Friedrich and Holtz, 2010). Cows were considered pregnant if serum PAG levels 33 days after insemination exceeded 2 ng/mL.

To assess to what extent pregnancy rate depends on the stage of the estrous cycle at which the cosynch protocol is initiated, based on progesterone concentrations 11 days before initiation of the cosynch protocol and on the day of initiation, the cycle was divided up as proposed by Cartmill et al. (2001): Stage at which progesterone level is below 1 ng/mL (“Low”, Days 1–4 and 19–21 of the cycle) and stage at which progesterone levels are higher than 1 ng/mL (“High”, Days 5–18 of the cycle) (Fig. 2). Thus, the following groups were formed: Cows in which treatment commenced while progesterone levels were on the rise (“Low-High”; Days 9–15 of the cycle at the beginning of the protocol), cows in which treatment commenced while progesterone level was high (“High-High”; Days 5–8 and 16–18), cows in which treatment commenced while progesterone levels were on the decline (“High-Low”; Days 19–21 and Days 1–4) and non-cycling cows (“Low-Low”). Pregnancy rate of cows treated with hCG twice (at the beginning and at the end of the cosynch protocol) the year before was compared to that of cows not having been exposed to hCG, to determine whether repeated

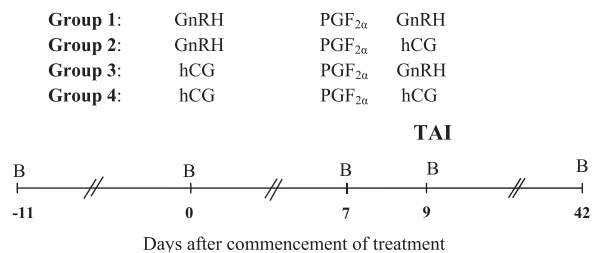


Fig. 1. Treatment schedule indicating the four treatment regimens constituting a classic cosynch protocol in which hCG was substituted for GnRH at the beginning, at the end or at beginning and end as well as days at which blood samples were drawn (B) and when cows were inseminated (TAI).

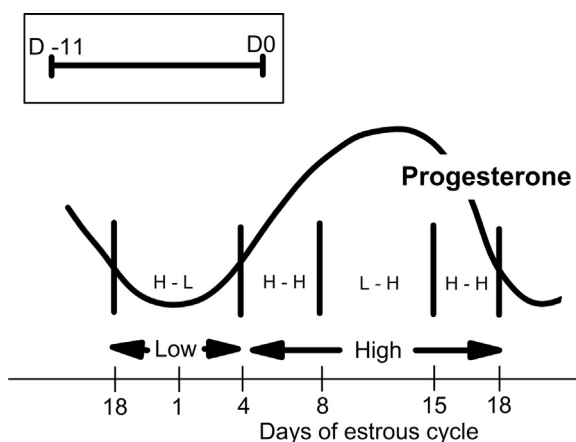


Fig. 2. Phases of the estrous cycle when treatment was initiated. Depending on serum progesterone concentrations 11 days before commencement (D – 11) and on the day of commencement of the protocol (D 0) cows were assigned to Groups high-high (H-H), high-low (H-L), low-high (L-H) and low-low (L-L). Day 1 of the cycle was the first day of standing estrus. The boxed-in line represents the 11 day interval between Day – 11 and Day 0.

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