J. Dairy Sci. 99:1–16
http://dx.doi.org/10.3168/jds.2016-11247

Physiological predictors of ovulation and pregnancy risk in a fixed-time artificial insemination program[1]

J. S. Stevenson[2]
Department of Animal Sciences and Industry, Kansas State University, Manhattan 66506-0201

ABSTRACT

The objective of this study was to determine the relative importance and contribution of several physiological factors as predictors of pregnancy risk in an Ovsynch (GnRH-1 – 7 d – $PGF_{2\alpha}$ – 56 h – GnRH-2 – 16 h – artificial insemination) timed artificial insemination program: (1) age of the corpus luteum (CL; original CL, new CL, or both in response to GnRH-1) and resulting progesterone concentrations as they affected luteolysis, ovulation after GnRH-2, and pregnancy risk; (2) progesterone concentration before GnRH-1 and GnRH-2 on subsequent ovulatory response to GnRH-1 and GnRH-2 as well as pregnancy risk; and (3) a combination of these factors in a multivariable logistic regression model to predict pregnancy risk. Original data from 7 published studies were combined including ovulatory responses to both GnRH injections, blood progesterone concentrations before GnRH-1, before $PGF_{2\alpha}$, and at 48 h after $PGF_{2\alpha}$, pregnancy per artificial insemination at d 32 and 60 after artificial insemination, and intervening pregnancy loss. Ovulation outcomes were greater at lesser progesterone concentrations after both GnRH injections despite the fact that pregnancy outcomes were greatest when progesterone exceeded 3 ng/mL before GnRH-1 and $PGF_{2\alpha}$, suggesting that greater progesterone concentration before GnRH-1 and incidence of ovulation act via different mechanisms to improve subsequent fertility. Ovulation after GnRH-2 and subsequent pregnancy outcome were positively related to lesser concentrations of progesterone at 48 h after $PGF_{2\alpha}$; however, for maximal pregnancy outcome, progesterone should be <0.5 ng/mL. Cows with a CL that also formed a new CL after GnRH-1 had the greatest pregnancy outcome, whereas cows with only a new CL (i.e., anovulatory cows and those at or near estrus without a CL at GnRH-1) had the poorest pregnancy outcome. Pregnancy loss was not affected by CL status during the timed artificial insemination program. Receiver operator curves produced cut points of progesterone concentration that predicted ovulation and pregnancy risk. Selected cut points of progesterone concentration 48 h after $PGF_{2\alpha}$ produced true positive risks greater than 90% and false positive risks less than 25%, thus demonstrating the high predictability of ovulation after GnRH-2 and subsequent pregnancy outcome based on progesterone concentration. We conclude that progesterone concentration is highly predictive of ovulation and pregnancy risk in addition to body condition, size of ovulatory follicle, and parity.
Key words: luteolysis, ovulation, progesterone, pregnancy risk

INTRODUCTION

Since the first report of a practical and successful fixed-time artificial insemination (**TAI**) program (i.e., Ovsynch: GnRH-1 – 7 d – $PGF_{2\alpha}$ – 56 h – GnRH-2 – 16 h – TAI; Figure 1) applied to lactating dairy cows on a commercial dairy farm (Pursley et al., 1997), physiological responses of the hypothalamo-pituitary-ovarian axis to exogenous hormonal interventions of the Ovsynch procedure have been studied. Application of Ovsynch and various adaptations of this program are now extensively used worldwide in the beef and dairy cattle industry (Wiltbank and Pursley, 2014). Preliminary studies formed the foundation for developing bovine TAI programs when GnRH administered to control follicular waves was followed in 6 d (Twagiramungu et al., 1995) or 7 d (Thatcher et al., 1989) by $PGF_{2\alpha}$ to regress functional luteal tissue in a coordinated fashion (Pursley et al., 1997). Lactating dairy cows treated with the Ovsynch program beginning on d 5 through 12 of the estrous cycle had greater incidences of ovulation and pregnancy per AI (**P/AI**) than cows treated at random stages of the estrous cycle (Vasconcelos et al., 1999).

On the basis of the hypothesis that fertility after a TAI program was related to the stage of the estrous cycle at which it was initiated (i.e., stage of the first

Received March 31, 2016.
Accepted August 18, 2016.
[1]Contribution number 16-286-J from the Kansas Agricultural Experiment Station, Manhattan 66506.
[2]Corresponding author: jss@ksu.edu

Figure 1. Standard 7-d Ovsynch timed AI program. GnRH (100 μg) = GnRH-1 or GnRH-2; $PGF_{2\alpha}$ (25 mg); TAI = timed AI. Color version available online.

ovarian follicular wave), presynchronization of estrous cycles resulting in a larger proportion of cows in diestrus at the onset of the TAI program produced greater P/AI than cows initiating the TAI programs at random stages of the estrous cycle (Moreira et al., 2001; Cartmill et al., 2001; El-Zarkouny et al., 2004). Overall, lactating dairy cows exposed to the $PGF_{2\alpha}$-presynch program have 42% greater odds of pregnancy compared with cows receiving only the TAI program (Bisinotto et al., 2014). Therefore, understanding the limitations and potential enhancements of the standard Ovsynch program is critical to maximizing its success.

At least 3 obvious factors are commonly thought to limit the success of the Ovsynch program including (1) initiating a new follicular wave by inducing ovulation of a dominant follicle in response to GnRH-1; (2) inducing luteolysis of all luteal structures in response to $PGF_{2\alpha}$ as evidenced by concentrations of progesterone <0.4 ng/mL at TAI (Santos et al., 2010; Wiltbank et al., 2014); and (3) timely ovulation of the preovulatory follicle in response to GnRH-2 to coincide with fixed-time semen placement in the uterus (Pursley et al., 1998) to maximize conception.

In the absence of any presynchronization treatment, synchronization of follicle growth by inducing ovulation in response to GnRH-1 of the TAI program occurs in only 45 to 50% of dairy cows (Pursley et al., 1995; Moreira et al., 2001). In addition, approximately 50 to 66% of dairy cows (Pursley et al., 1995; Vasconcelos et al., 1999) at various stages of the estrous cycle ovulate in response to GnRH. Ovulation (Stevenson et al., 2008; Stevenson and Pulley, 2016) and GnRH-induced LH release are suppressed in the presence of a CL and larger concentrations of progesterone in dairy cattle (Giordano et al., 2012; Pulley and Stevenson, 2015).

A second limitation is the inability of a single dose of $PGF_{2\alpha}$ to induce complete regression of all luteal structures depending on the number and age of the CL. Corpora lutea less than 10 d old are often resistant to regression after a standard dose of $PGF_{2\alpha}$ (Momont and Seguin, 1984). Administering PG as a single dose on d 7 or as 2 doses on d 5 and 6 after GnRH-1 (5-d Ovsynch) usually results in 70 to 84% of cows with progesterone <0.3 ng/mL on the day of the TAI (Santos et al., 2010), and likely influences the characteristics of GnRH-induced LH release when progesterone concentrations are at or near baseline at the final GnRH treatment and subsequent P/AI (Stevenson and Pulley, 2016). Increased dose (Giordano et al., 2013; Wiltbank et al., 2015) or frequency of $PGF_{2\alpha}$ treatments (Brusveen et al., 2009; Santos et al., 2010; Ribeiro et al., 2012) at the end of Ovsynch program has enhanced luteolysis, reduced progesterone concentrations at TAI, and in some cases increased P/AI compared with a single standard dose (Santos et al., 2010; Ribeiro et al., 2012; Wiltbank et al., 2015).

A third limitation is the inability to synchronize ovulation between 24 and 32 h after GnRH-2 (Pursley et al., 1995). Synchronized ovulation averages 85% in dairy cows (Santos et al., 2010) and this percentage seems to decrease when cows are exposed to heat stress because of the supposed deleterious effects of hyperthermia on ovulation (López-Gatius et al., 2005). Multiple studies indicate that timing of GnRH-2 relative to $PGF_{2\alpha}$ and insemination is optimal for pregnancy risk when GnRH-2 is administered at approximately 56 h after $PGF_{2\alpha}$ and insemination occurs 12 to 16 h later (Stevenson, 2016b).

Of the 3 previously defined limitations, 2 are related to the success of ovulation, which is dependent on GnRH-induced LH release and its in vivo pre-GnRH steroid milieu (Stevenson and Pulley, 2016). To better understand and define the limitations of the standard Ovsynch program, we combined data from our published studies to examine the interrelationships of these factors. Of these limiting factors, we examined the influence of (1) age of the CL and resulting progesterone concentrations on luteolysis, ovulation response after GnRH-2, synchronization, and pregnancy risks; (2) progesterone concentration before GnRH-1 and GnRH-2 on subsequent ovulatory response to GnRH-1 and GnRH-2 and pregnancy risk; and (3) a combination of these factors in a multivariable logistic regression model used to predict pregnancy risk. Our objective was to determine the relative importance and contribution of these physiological factors as predictors of pregnancy risk in an Ovsynch TAI program.

MATERIALS AND METHODS

Source of Data

Data were combined from 7 published studies in which Ovsynch was applied to lactating dairy cows from 2004 through 2014.

Study 1. The first study involved lactating Holstein cows (n = 635) located in 6 Midwestern states (Stevenson et al., 2008). Estrous cycles were presynchronized with 2 injections of $PGF_{2\alpha}$ 14 d apart and cows were