

Superovulation: Advancements in cattle

Sapna Pal

Abstract

The major limiting factor to the development of embryo transfer technique in cattle is the variability in ovulatory response to FSH-induced superovulation. Protocols for the superovulation vary from one species to another and even in the same species of different strains. The advancement in the protocols of superovulatory treatment is highly evolved in past 50 to 60 years. The result of superovulation can be affected by type, source and half life of gonadotropin used. Various hormones like estradiol and progesterone are used to manipulate follicular waves which cause reduction in the variations caused by treatment of the cattle at the different stages of the follicular development. It has also been suggested that small antral follicles can be induced to grow by extending the treatment protocol.

Keywords: antral follicles, estrogen, estrous cycle, follicular wave, FSH, gonadotropin, progesterone, superovulation

Introduction

The superovulation is the technique to induce multiple ovulations in one ovulatory cycle by administering exogenous hormones (gonadotropins). According to a report from a total of 2948 beef cows, averages of 11.5ova/embryo with 6.2 transferable embryos were collected from each cow [1]. However, there was the great variability in both the superovulatory response and embryo quality. This high unpredictability in the superovulatory response is the major problem which affects the efficiency and profitability of embryo transfer programs [2].

The gonadotropins are usually administered before the estrus to induce multiple ovulations and after the injection of is hCG given at the onset of the estrus to induce ovulation. Although the studies were conducted on the usage of pituitary gonadotropins from the pigs, sheep, horse and cattle [3], but the use of eCG or PMSG hormones have become standard approach. Hafez et al. [4] developed a protocol used in the cow where 2000-3000 IU of eCG was administered on day 16 of the ovulatory cycle, followed by 10mg of estradiol-17 β on day 19 and 20 and 2000 IU of hCG on day 21. This became the standard protocol used at that time.

Gonadotropins in current superovulation treatment protocol

eCG is initially used in superovulation, but due to the recent researches the protocols have been changed rapidly. In 1978 Elsdon et al. [5] reported that treatment with crude pituitary extract containing FSH in combination with 20% LH results in the greater superovulatory response as compared to eCG. Studies of Monniaux et al. [6] also reported that ovulation rates and percentage of cows with more than 3 transferable embryos tends to be greater with the use of pituitary derived extract containing FSH than eCG. Other studies have not found much difference

between the pituitary extract containing FSH and eCG [7,8]. One of the reasons for the decrease in the ovulation and fertilization rates [9] by the use of eCG may be the frequent abnormal profiles of LH and progesterone in the animals treated with the eCG as revealed by endocrine studies[10,11].

Nowadays cattle are superstimulated using pituitary extracts containing FSH. The crude pituitary extract contains large amount of LH. Hence, there is a considerable variation in the FSH and LH content in the crude gonadotropin preparation [12]. The effects on the FSH and LH ratio of eCG have been examined on the superovulatory response and positive correlation has been found between the superovulatory response and FSH/LH ratio. Low ratios of FSH/LH activity resulted in decrease in the ovulatory response in immature rats and LH when added to eCG resulted into reduced superovulatory response in cattle. Superovulatory response have been reported to improve when pituitary extracts with low LH contamination are used in cattle [13, 12].

Sakaguchi et al. [14] examined the effect of epidural administration via caudal vertebrae on *in vivo* embryo production and *in vitro* embryo production followed by Ovum pick-up *in vitro* fertilization (OPU-IVF) in Japanese black cow. They concluded that single epidural FSH administration via the caudal vertebrae induces superovulation in Japanese black cows; it also appeared to improve embryonic development after OPU-IVF.

Ovarian follicular wave dynamics and superovulation

Follicular growth in cattle occurs in waves throughout the estrus cycle. When a cow ovulates, the follicle left is termed as corpus hemorrhagicum and after some days this corpus hemorrhagicum becomes corpus luteum and secretes progesterone. During this time due to increase in the FSH the follicular wave proceed. But, the dominant follicle does

not ovulate due to presence of corpus luteum secreting progesterone as a result it become nonfunctional. After the first dominant follicle become nonfunctional there is an increase in the FSH and a second wave of follicle begin to grow [15], during this time the dominant follicle will only proceed to ovulation if its growth coincides with regression of corpus luteum caused by the release of prostaglandin and subsequent decrease in progesterone. Some cattle have two waves before ovulation and some have three waves of follicle before ovulation.

The conventionally used protocol to start superovulation procedure during mid cycle was based on the experimental information in which it is reported that when superstimulatory treatment were initiated 8 to 12 days after estrus then there is a greater superovulatory response [16]. With the help of ultrasonography it is now known that 7 to 11 days after ovulation is the approximate time when second follicle wave emerges [17] and at that time a group of growing follicles would be present. However, the time of second follicular wave emergence have been shown to differ between the animals having two wave cycles and 3 wave cycles. It is shown that when gonadotropin treatments are initiated a few days before or after the start of second follicular wave the superovulatory responses are reduced as compared to the day of wave emergence [18, 19, 20].

Approach to synchronize follicular waves

GnRH- studies have been shown that after the induction of ovulation by GnRH a new follicular wave starts approximately 1.5-2 days after the treatment. However, emergence of follicular wave occurs only when gonadotropin releasing hormone induces ovulation [21] and according to recent studies administering GnRH at random stages of estrous cycle results in ovulation in 44% to 54% dairy cows [22,23], 56% of beef heifers [21] and 60% of beef cows [24]. Therefore, for the superstimulatory treatment the interval from GnRH treatment to wave emergence might be too inconsistent.

In recent practices a progestin device is inserted at random stages of the estrous cycle and GnRH hormone is administered 2 or 3 days later and after that superstimulation treatment begin which usually started after 1.5 to 2.5 days of GnRH administration. The reason of synchronization of follicular waves emergence and ovulatory response improvement might be because of presence of the progestin device that has prevented ovulation of mature follicle thus increasing the efficacy of GnRH.

Estrogen and progesterone- superstimulation can be initiated without regard to the stage of estrus cycle if the follicular wave is electively induced. It also eliminates the 8 to 12 days wait to initiate gonadotropin treatment [25]. Estradiol and progesterone induces synchronous emergence of follicular wave. This approach have been studied and reviewed extensively. Practitioners all around the world have also recently included this approach into protocol that permits fixed-time AI (FTAI) of the donors [26, 27].

Exogenously induced follicular waves- this procedure is under studies but recently investigators have shown that lengthening the superstimulatory protocol from the normal 4 day to 7 days recruit more follicles into the wave and provide the time required for them to acquire the capacity to ovulate [28].

In recent researches the 7-day FSH treatment protocol produces 2.5 times more transferable embryos per animal than the normal treatment after the ultrasound-guided oocyte collection and in vitro embryo production [29]. Overall, the prolonged FSH treatment protocol might be effective in recruiting small follicles into the follicular cohort available for superstimulation. It also gives follicles additional time to reach ovulatory size and ovulate which might not be provided in the traditional 4-day superstimulatory treatment protocol.

Recent superstimulatory practices and their responses

The experiment conducted by Barros et al. [30] shows that if Nelore cows were superstimulated with FSH for over 3 days and the last two FSH injections (in fourth day) were replaced by two injections of 200 IU eCG then the number of ova/embryo and transferable embryos increases significantly. In most of the studies that shows the effect of the eCG treatment the donor of the control groups are found to produce average to below average transferable embryos. However, the experiment conducted by Davis et al. [31] shows above average production of transferable embryos in the control group, suggesting that this treatment might be helpful during the depressed embryo production. In most recent study Hackbart et al. [32] reported that the elevation of circulatory insulin produced by administering propylene glycol (PROP) for every 4h or LH during the last few days of follicular development has little or no effect on the embryo quality and percentage of embryos that degenerates during first week after ovulation. However, these treatments reduce the percentage of superstimulated follicles that ovulated without altering the superstimulatory response to FSH treatment.

Conclusion

Superovulation treatment plays very important role in the embryo transfer technique and a lot of efforts have been put in improving and modifying the protocols. Studies have shown that variability in the superovulatory response is mainly due to the differences in the follicular population of the donor animal. By examining the responses of different protocols on different cattle, the most suitable and effective protocol can be selected.

Progress in the advancement of recombinant technology will soon provide us different types of recombinant gonadotropins (eg. recombinant FSH) which will be more effective than the conventionally used gonadotropins and will further increase the superovulatory response and embryo quality. Administering the eCG on different time intervals of FSH treatment might be useful in increasing number of transferable embryos. Gonadotropins extracted from a particular species of animal might also possibly increase the superovulatory responses when administered to a particular type of species. Hormones other than gonadotropins can also affect superovulatory treatment and with the help of thorough study of their actions, positive or negative effects of these hormones can be detected.

References

- [1] Looney C, Bondioli K, Hill K, Massey J. Superovulation of donor cows with bovine follicle-stimulating hormone (bFSH) produced by recombinant DNA technology. *Theriogenology* 1988; 29:271.
- [2] Rogan D, Strauss C, Mapletoft R, Bó G, Tribulo H, Szkudlinski M, et al. Opportunities for the production of recombinant gonadotropins for assisted reproduction and embryo transfer. In: *Proceedings of the Joint Annual Meeting of the Canadian and American Embryo Transfer Association, Montreal, Quebec; 2009; 59–67.*
- [3] Gordon I. Problems and prospects in cattle egg transfer. *Irish Vet J* 1975; 29:21–62.
- [4] Hafez ES, Sugie T, Hunt WL. Superovulation and related phenomena in the beef cow: effect of oestrogen administration on production of ova. *J Reprod Fertil* 1963; 5:381–8.
- [5] Elsdon R, Nelson L, Seidel G. Superovulation of cows with follicle stimulating hormone and pregnant mare's serum gonadotropin. *Theriogenology* 1978; 9:17–26.
- [6] Monniaux D, Chupin D, Saumande J. Superovulatory responses of cattle. *Theriogenology* 1983; 19:55–82.
- [7] Goulding D, Williams D, Roche J, Boland M. Factors affecting superovulation in heifers treated with PMSG. *Theriogenology* 1996; 45:765–73.
- [8] Mapletoft R, Pawlyshyn V, Garcia A, Bó G, Willmott N, Saunders J, et al. Comparison of four different gonadotropin treatments for inducing superovulation in cows with 1:29 translocation. *Theriogenology* 1990; 33:282.
- [9] Callesen H, Greve T, Hyttel P. Preovulatory endocrinology and oocyte maturation in superovulated cattle. *Theriogenology* 1986; 25:71–86.
- [10] Greve T, Callesen H, Hyttel P. Endocrine profiles and egg quality in the superovulated cow. *Nord Vet Med* 1983; 35:408–21.
- [11] Mikel-Jenson A, Greve T, Madej A, Edqvist L. Endocrine profiles and embryo quality in the PMSG-PGF2a-treated cow. *Theriogenology* 1982; 18:33–4.
- [12] Murphy B, Mapletoft R, Manns J, Humphrey W. Variability in gonadotrophin preparations as a factor in the superovulatory response. *Theriogenology* 1984; 21:117–25.
- [13] Murphy B, Martinuk S. Equine chorionic gonadotropin. *Endocrine Rev* 1991; 12:27–44.
- [14] Sakaguchi K, Ideta A, Yanagawa Y, Nagano M, Katagiri S, Konishi M. Effect of a single epidural administration of follicle-stimulating hormone via caudal vertebrae on superstimulation for in vitro and in vivo embryo production in Japanese black cow. *J Reprod Dev* 2018; 64:451-455.
- [15] Boer HMT, Röblits S, Stötzel C, Veerkamp RF, Kemp B, Woelders H. Mechanisms regulating follicle wave patterns in the bovine estrous cycle investigated with a mathematical model. *J Dairy Sci* 2011; 94:5987-6000.
- [16] Bó GA, Adams GP, Pierson RA, Mapletoft RJ. Exogenous control of follicular wave emergence in cattle. *Theriogenology* 1995; 43: 31–40.
- [17] Ginther OJ, Knopf L, Kastelic JP. Temporal associations among ovarian events in cattle during oestrous cycles with two and three follicular waves. *J Reprod Fertil* 1989; 87:223–30.
- [18] Armstrong D. Recent advances in superovulation of cattle. *Theriogenology* 1993; 39:7–24.
- [19] Adams GP, Nasser LF, Bó GA, Garcia A, Del Campo MR, Mapletoft RJ. Superstimulatory response of ovarian

follicles of Wave 1 versus Wave 2 in heifers. *Theriogenology* 1994; 42:1103–13.

[20] Nasser L, Adams GP, Bó GA, Mapletoft RJ. Ovarian superstimulatory response relative to follicular wave emergence in heifers. *Theriogenology* 1993; 40:713–24.

[21] Martinez MF, Adams GP, Bergfelt D, Kastelic JP, Mapletoft RJ. Effect of LH or GnRH on the dominant follicle of the first follicular wave in heifers. *Anim Reprod Sci* 1999; 57:23–33.

[22] Bello NM, Steibel JP, Pursley JR. Optimizing ovulation to first GnRH improved outcomes to each hormonal injection of Ovsynch in lactating dairy cows. *J Dairy Sci* 2006; 89:3413–24.

[23] Colazo MG, Gordon MB, Rajamahendran R, Mapletoft RJ, Ambrose DJ. Pregnancy rates to timed artificial insemination in dairy cows treated with gonadotropin-releasing hormone or porcine luteinizing hormone. *Theriogenology* 2009; 72:262–70.

[24] Small JA, Colazo MG, Kastelic JP, Mapletoft RJ. Effects of progesterone pre-synchronization and eCG on pregnancy rates to GnRH-based, timed-AI in beef cattle. *Theriogenology* 2009; 71: 698–706.

[25] Mapletoft RJ, Bennett-Steward K, Adams GP. Recent advances in the superovulation of cattle. *Reprod Nutr Dev* 2002; 42:601–11.

[26] Bó GA, Baruselli PS, Chesta PM, Martin CM. The timing of ovulation and insemination schedules in superstimulated cattle. *Theriogenology* 2006; 65:89–101.

[27] Baruselli P, Sá Filho M, Martins C, Nasser L, Nogueira M, Barros C, et al. Superovulation and embryo transfer in *Bos indicus* cattle. *Theriogenology* 2006; 65:77–88.

[28] García Guerra A, Tribulo A, Yapura J, Singh J, Mapletoft R. Lengthening the superstimulatory treatment protocol increases ovarian response and number of transferable embryos in beef cows. *Theriogenology* 2012; 78:353–60.

[29] Dias FC, Dadarwal D, Adams GP, Mrigank H, Mapletoft RJ, Singh J. Length of the follicular growing phase and oocyte competence in beef heifers. *Theriogenology* 2013; 79:1177–83.

[30] Barros CM, Barcelos AC, Gouvea LM, Meneghel M, Barcelos DS, Barcelos LN, et al. Improvement of a superovulatory protocol in Nelore cows: replacing the last two doses of pFSH by eCG. *Reprod Fertil Dev* 2008; 20:152.

[31] Davis RL, Arteaga A, Hasler JF. Addition of equine chorionic gonadotropin to a traditional follicle stimulating hormone protocol for superovulation of *Bos taurus* beef cows. *Reprod Fertil Dev* 2012; 24:224–5.

[32] Hackbart KS, Bender RW, Carvalho PD, Vieira LM, Dresch AR, Guenther JN et al. Effects of propylene glycol or elevated luteinizing hormone during follicle development on ovulation, fertilization, and early embryo development. *Biolre* 2017; 97(4):550-563.

