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Review Article

Superovulation in Cows: A Review

Sakine Ülküm Çizmeci[1*] and Mehmet Güler

Department of Obstetrics and Gynecology, Faculty of Veterinary Medicine, Selcuk University, Turkey
***Corresponding author:** ulkum@selcuk.edu.tr

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ABSTRACT

Ovulation is release of secondary oocyte from ovary upon ovulation of grown and maturated Graafian follicle. Superovulation is the bunch of processes to have the female to produce more eggs than the normal count of its species in each estrus by gonadotropin and to increase the number of embryos. Ten or more live eggs can be collected in each estrus from appropriate superovulated cows and heifers. Approximately 5 transferrable embryos can be collected upon 85% of superovulated normal fertile donors. Usually FHS and PMSG and rarely hMG are used for superovulation of cows. LH, hCG, GnRH, estradiol 17-β, prostaglandins, Anti-PMSG, gestagens and inhibin are used as auxiliary to the superovulator hormones. 10 or more oocytes can be collected in an estrus from the cow appropriately superovulated. As the repetition frequency of superovulation increases chance for procurement of alive embryo decreases.

Key words: Cow, FSH, PMSG, Superovulation

INTRODUCTION

Ovulation is release of the secondary oocyte grown and maturated from the Graafian follicle. LH (Luteinizing Hormone) released from the anterior lobe of the pituitary gland is the factor starts ovulation. Release of oocyte is induced with effect of LH release approximately 24 hours prior to ovulation in cow, dog, goat and sheep (Çolak, 2010).

Superovulation allows producing a greater number of oocytes and embryos than the normal cycle in female animals (Maarten,2000, Ayasan and Karakazak, 2010, Hızlı *et al.*, 2011). Ten or more live oocytes can be collected in each estrus from superovulated cows and heifers. Approximately 5 transferable embryos can be collected upon 80-85% of superovulated normal fertile donors. The main principle of superovulation is to provide follicular stimulation by administration of FSH and similar hormones (Kanagawa *et al.*, 1995, Hussein *et al.*, 2014).

A newborn female calf has almost 150.000-200.000 primordial follicles in her ovaries (Noakes, 2001). In a study, sum of primordial, primary, secondary and Graafian follicles was 77.169 in a 4-year-old cow (Gordon, 2003). Almost 200 follicles grow in every estrus in a heifer in puberty (Noakes, 2001). It is reported that number of ovulated follicles can't to exceed 300 in her lifetime in a cow (Gordon, 2003). Superovulation if can be applied together with embryo transfer gives the chance to use to the oocytes which do not have not economic potential (Hızlı *et al.*, 2011). For that purpose, generally, FSH (Follicle Stimulating Hormone) and less commonly PMSG (Pregnant Mare Serum Gonadotropin) hormones are used. FSH is obtained from pig and sheep pineal gland extract while PMSG is obtained from serum of pregnant mares (Akyol *et al.*, 2004).

Response of animals to superovulation depends on race, age and general condition, type of hormone, way of administration, climate, nutrition, and environmental (Kafi and McGowan, 1997, Mapletoft *et al.*, 2002, Silva *et al.*, 2009, Ayasan and Karakazak, 2010, Hızlı *et al.*, 2011, Hussein *et al.*, 2014). The ovarian response of each female is attached to the count of gonadotropin sensitive follicles existent at the time application is started (Hussein *et al.*, 2014).

Superovulatory hormones and their usage

Usually, there are used FSH, PMS for superovulation of cows, whereas hMG (Human Menopausal Gonadotropin) is used less commonly (Akyol, 2001, Bowen, 2003). Advantages of using PMSG are that it is cheaper and easier to obtain than FSH and the single dose is enough. Disadvantages are that there is a huge variation in the quality of the collected embryos and has residue problem after administration (Akyol, 2001).

FSH: This hormone is called as follicle stimulating hormone and produced in gonadotrope cells located in the anterior lobe of the pituitary gland (Yilmaz, 1999). FSH is necessary for follicle development, antrum formation and

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estrogen release (Alacam, 2010). FSH stimulates the release of primarily β-estradiol and other estrogens from theca interna and granulosa cells of growing follicles. FSH and LH act together to make follicles release estrogen. FSH and LH act synergistically until ovulation. Estrogen hormone helps FSH in follicle development. As blood estrogen level increase FSH release decreases and LH becomes effective (Yilmaz, 1999, Akyol, 2001). FSH is induced expression of significant genes involved in follicle maturation and the generation of LH receptors (Sidi *et al.,* 2016). FSH stimulates follicle development by interacting with specific receptors on granulosa cells. Such receptors are imperative for the fulfilment of follicle maturation and to consent ovulation to be formed in response to the preovulatory wave of LH (Lehloenya *et al.*, 2008).

FSH is a pituitary gonadotropin which is usually obtained from horse, pig and sheep. Application dose is 25-50 mg depending on live weight of cow (Akyol, 2001, Tekeli, 2010). The half-life of FSH is approximately 2 hours thus it must be administered in repeated doses. The biological half-life of FSH is short; hence it is administered two doses in a day (Kimura *et al.*, 2007).

Researchers reported that the best time to start FSH administration is the 9th – 14th days of the cycle (Kanagawa *et al.,* 1995). The reasons for the hormone to be effective around those days are both the presence of a mature CL; also, it is the best time of follicular wave for beginning superovulation applications (Akyol, 2001).

Researchers reported that they administered FSH in a single dose subcutaneously or in decreasing doses (Table 1) for 4 days (Bo *et al.*, 1994, Alvarez *et al.,* 2010). As a result of these studies they have been indicated that there was no evident difference in superovulation response and single dose FSH administration is very handy. In addition, some researchers gave a single injection of FSH into epidural space to reduce workload and error. As a result of, they reported that similar transferable embryo rate was obtained (Imron *et al.*, 2016).

PMSG: PMSG is known as pregnant mare serum gonadotropin but recently it is more called as eCG (Equine chorionic gonadotropin) in terminology (Bowen, 2003). It is a placenta originated gonadotropin which is obtained from serum of pregnant mare (Tekeli 2010). It mostly has FSH-like effects and besides has LH-like effects as well (Akyol, 2001).

PMSG is used between doses of 2000 IU and 4000 IU in cows. The single administration (Table 2) is enough because it has a long half-life (Tekeli, 2010). Single dose application of PMSG is preventing the stress of injection in cow and is decrease labour. However, due to its long halflife, there may be big follicles remained in ovary even after superovulation. Estrogen released from those follicles may have the adverse effect on the number of embryos collected (Akyol, 2001). One main disadvantage of PMSG is caused formation of antibody and ovarian cysts in donor animals (Tekeli, 2010).

Estradiol level does not have the strong adverse effect on number and quality of embryos collected during the early period of embryonic growth. But it may have some adverse effects such as nucleus abnormalities on embryos in the later growth period. This negative situation can be resolved by using anti-PMSG agents (Kanagawa *et al.*, 1995).

hMG: This hormone was named as hMG (human menopausal gonadatrophin) which was obtained from urine of a woman with menopause upon idea of it may use for the superovulation. Researchers reported that there was no positive and sufficient result in studies about hMG in contrast to studies about FSH and PMSG (Macun and Kaymaz 2006).

hCG or LH usage for stimulation of ovulation: hCG is released by cincitiotrophoblas cells in chorionic villus of the pregnant woman (Akyol, 2001). It reaches peak level in the 70th day of pregnancy and decreases gradually until birth (Yilmaz, 1999). Ovulations distribute over 24-48 hours or more time in superovulated cows thus causes problems in ovum fertilization. hCG (2500-5000 IU) administration at the beginning of estrus may preferred in order to avoid that problem (Alaçam, 2010).

LH is known as luteinizing hormone. It is synthesized by gonadotroph cells in the anterior lobe of pituitary gland and released. Normally, ovulation occurs 24 hours after LH peak in cows. It has role in follicle development and maturation in females. Follicles must be stimulated by FSH initially for this effect to take place. Luteinizing hormone provides ovulation of mature follicle. Plasma LH level peaks just before ovulation thus LH is accepted as the stimulation that starts ovulation (Akyol, 2001).

Hormones that used together with superovulatory hormones

GnRH: Depending on period of cycle and follicular growth in ovary (follicular waves), GnRH hasten growth of young follicles, ovulated follicles which are rich in estrogen (antral follicles) and luteinizing big or old follicles (Dinc, 2006). It is reported that GnRH administration during AI or between 11st – 14th days increase pregnancy rates. The efficiency of GnRH treatment, prevention of embryonic deaths, controlling follicular growth, synchronization program that used PGF2α for luteinization depend on postpartum stimulation of ovulation (Jadov *et al.,* 2010). GnRH is used in cases where hCG is indicated (Akyol 2001)

Estradiol 17-β: Estrus signs are sometimes weak in cows administered superovulation. Therefore, 10 mg estradiol 17-β may inject one day before expected estrus (Dinc, 2006). However, estradiol administration which is a mostly used for synchronization of follicular wave occurrence for superovulation cannot be used in many countries due to side effects and the negative influence on human health of estrogens (Hussein *et al.*, 2014).

Prostaglandins: The major development in embryo transfer technology was start of using prostaglandins which have strong luteolytic effect. Prostaglandins are used to increase the efficiency of superovulation in donor animals, and to provide sexual synchronization between donor and recipient animals. Single injection of PGF2α for the animals in diestrus phase is done in order to synchronize the sexual cycles. Also, PGF2α may apply twice 9-11 days interval for the animals group for the same reason (Alaçam, 2010).

Table 1: FSH based superovulation program in cattle (Maarten, 2000).

Application days	Applications
-27	5 cc $PGF_2\alpha$ (IM)
-17	5 cc $PGF_2\alpha$ (IM)
-14	Oestrus
am	50 mg (2.5 cc) FSH (IM)
-4 pm	50 mg (2.5 cc) FSH (IM)
am	50 mg (2.5 cc) FSH (IM)
-3 pm	50 mg (2.5 cc) FSH (IM)
am	50 mg (2.5 cc) FSH + 30 mg $PGF_2\alpha$ (IM)
-2 pm	50 mg (2.5 cc) FSH + 25 mg $PGF_2\alpha$ (IM)
am	50 mg (2.5 cc) FSH (IM)
-1 pm	50 mg (2.5 cc) FSH (IM)
0	Oestrus – Artifical Insemination
7	Embryo Collection

Artifical insemination is to be 4-6 hours after onset of estrus and repeated after 10-12 hours. IM: intramuscular, am: morning, pm: evening.

Table 2: Superovulation program in cows with PMSG administration (Maarten 2000).

Days	Application 1 (4 Days)	Application 2 (5 Days)
-18	25 mg $PGF_2\alpha$	25 mg $PGF_2\alpha$
-15	Oestrus	Oestrus
-5 am		2500 IU PMSG
-4 am	2500 IU PMSG	
-2 am	35 mg $PGF_2\alpha$	35 mg $PGF_2\alpha$
pm	20 mg $PGF_2\alpha$	20 mg $PGF_2\alpha$
0	Oestrus-Artifical Insemination	Oestrus- Artifical Insemination

Artifical insemination is to be 4-6 hours after onset of estrus and repeated after 10-12 hours, am: morning, pm: evening.

Prostaglandin is injected at the 48th hour after FSH and PMSG administration for superovulation of cows. Administered natural prostaglandins twice (in the morning and afternoon) totally 30 mg daily or the single administrated of its analogues is enough. Estrus starts at 42nd – 48th hours following prostaglandin administration. AI is performed 10-24 hours after onset of estrus (Akyol, 2001).

Anti-PMSG: It can be obtained from animals such as turkey, rabbit, sheep and goat and used to remove PMSG residues present in serum. Residual PMSG has the adverse effect on the number of transferable embryos. Thus, anti-PMSG may be administered 18-24 hours after the beginning of estrus in order to neutralize the residual quantity of PMSG in cows. Anti-PMSG is administered shortly after LH peak prior to ovulation thus providing increase in number of transferrable embryos (Kanagawa *et al.,* 1995). Besides, occurrence of big follicle and cyst cases is decreased by anti-PMSG administrations (Akyol, 2001).

Gestagens: Progesterone restricts the release of GnRH hormone from the hypothalamus and reduces FSH and LH release from the anterior lobe of pituitary gland. The absence of ovulation during pregnancy is explained by the decrease in LH release (Yilmaz, 1999). It is possible to shorten superovulation intervals by using progestogenic agents. Its two main purposes are;

- To increase the number of embryos in good quality obtained upon use of progestogenic agents together with prostaglandins. PRID or other implants can be used for this.
- To prevent dominant follicle formation by modifying endocrinological composition to be stable to that during luteal phase (Akyol, 2001).

Inhibin: FSH release from the pituitary gland is inhibited and serum FSH level drops immediately after inhibin injection (Senger, 1999). Thus, it has been concluded that inhibin is effective in the regulation of FSH release and production. According to this information, there are some studies about providing growth of small and middle-sized follicles and increasing superovulation efficiency (Akyol, 2001).

Follicular wave and superovulation

Two, 3 and sometimes 4 follicular waves occur in the luteal phase, nominee a group of follicles starts maturate. More than one follicle start to grow and their diameter reach about 4-5 mm. Only one of those follicles outgrows after this stage. This follicle is also called dominant follicle. In this phase, dominant follicle prevents the growth of others and continues to grow alone (Bülbül *et al.,* 2010).

Follicular waves are controlled with luteal growth in cows that follicular and luteal growth is not understood. Therefore, approaches to handle together estrus and ovulation were developed (Dinc *et al.,* 2009).

Presence of dominant follicle prevents the growth of other follicles during superovulation studies as well hence the number of collected embryos is few. This situation does not happen in superovulation studies in which are performed before formation of dominant follicle (Akyol, 2001).

Repeated superovulation practices

The number of collected embryos usually decreases in repeated superovulations in cows. As the repetition frequencies of superovulation increase the chance to obtain embryo decrease (Lubbadeh *et al.,* 1980). As observed in the studies, if there are 40 or more days between superovulations, number and quality of obtained embryos does not affect adversely (Kanagawa *et al.,* 1995).

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