Reproductive Endocrinology of the Cow: Part 2 Superovulation

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Introduction

In Part I the intricate hormonal relationships involved in the bovine estrous cycle were described. The widespread growth of the embryo transfer industry has made manipulation of reproductive events of both donors and recipients a commonplace practice in reproductive programs. This article presents a review of the various methods used to produce a superovulatory response in cows and the basic endocrinological changes which allow this response to occur.

Superovulation can be defined as the increased ovulatory response induced in an individual above the normal range of ovulation rates for that individual. In cattle, therefore, three or more ovulations are considered as superovulatory responses (1).

Gonadotrophins used in Superovulation

Soon after the discovery of the pituitary gonadotrophins in 1927 (2,3), aqueous extracts of pituitary tissue resuspended in a physiological solution were used to stimulate follicular growth and multiple ovulation in animals (4).Casida and his coworkers were the first to report superovulation in cattle using pituitary extracts (5). Pregnant mares' serum gonadotrophin (PMSG) was discovered in 1930 (6). In 1968, the presence of gonadotrophins in the urine of postmenopausal women [human menopausal gonadotrophin (hMG)] was demonstrated (7). Pituitary gonadotrophins of porcine or equine origin (FSH), PMSG and hMG have now all been used extensively as superovulatory drugs in cows.

A. Pregnant Mares' Serum Gonadotrophin

PMSG is a glycoprotein found in high concentrations in the blood of pregnant mares between 46-130 days of gestation (6) and has been shown to possess both FSH and LH activities (8,9). For commercial preparations, PMSG is extracted from the serum collected from pregnant mares and is lyophilized to allow storage. Its potency is expressed in International Units (IU). Specific gonadotrophic activity in one IU equals 0.25 mg of a standard preparation held by the World Health Organization (Ayerst Laboratories, Montreal, Canada).

A number of reports indicate that the ovulatory response in animals varies with particular batch of the PMSG used for superovulation (10,11) and that the ratio of the FSH and LH activities differs among commercial batches of PMSG (12). In more recent reports, higher ovulation rates were recorded when the FSH activity in the PMSG was higher than the LH activity (8,13). Ovulation rate decreased when FSH/LH ratio was low.

Since PMSG contains more sialic acid, the half life of this gonadotrophin is longer than that of FSH or LH. The first order half lives of LH (human chorionic gonadotrophin), FSH and PMSG in cow's circulation were 13.8 min., 5h and 51.2h respectively (14,15,16). The administration of PMSG alone, during the transition from the luteal phase to the follicular phase, induced superovulation in cattle. However, variation in the time from PMSG treatment to the onset of estrus and low ovulation rates were major problems (17).

More recently, the availability of prostaglandin $F_2\alpha$ (PG) and its use in superovulation programs has improved the regulation of estrus in donors. It has been shown that superovulatory treatments (PMSG-single dose, FSH-multiple doses) initiated during the mid-luteal phase (days 9-13) of the estrous cycle result in higher ovulation rates than treatment begun in the early luteal phase (days 3-8) (18,19,20).

Treatment with PMSG towards the end of the follicular phase does not increase the number of ovulating follicles because during this phase of the cycle there is a reduction in the number of maturing follicles (4). It has been demonstrated that in spite of continuous growth and development of follicles, the ovaries of a cow usually contain one large estrogen active (EA) follicle from day 4 until the time of ovulation (21). As estrogen secretion from this follicle increases, the plasma concentration of FSH falls (22). In growing follicles, FSH is required to

equip the granulosa cells with sufficient aromatase activity to maintain estrogen synthesis. Since availability of FSH to smaller follicles is inhibited by the large EA follicle, these follicles fail to aromatise theca-derived androgens into estrogens (23). Such estrogen inactive follicles are atretic and contain smaller population of FSH and LH receptors (23). It appears, therefore, that poor ovulation rate results from irreversible atresia in the majority of smaller follicles when PMSG is administered towards the end of the follicular phase of the estrous cycle. Insufficient time for maturation of these follicles before the preovulatory gonadotrophic surge may also be an explanation.

Doses of PMSG varying from 800-3000 IU have been used successfully to induce superovulation in cows (24,25,26,27). Superovulation with PMSG requires only one injection since it has a long half life and subdivision of the total dose into multiple injections does not improve ovulation rate (26). During superovulatory treatment, estrus in donor cows is regulated by either intramuscular or intra-uterine administration of PG or its analogues. Although intra-uterine treatment of donors with PG is economic its availability at reasonable cost hardly justifies this route of administration (18). The normal dose of $PGF_{2}\alpha$ is in the range of 25-40 mg, administered either as one dose or as two doses 4-10 hours apart, 40 to 48 hours after the initiation of superovulation (18,28). This induces luteolysis in donor cows. Recent reports suggest that the administration of 2 doses of PG 12 hours apart produced optimal superovulatory response (29). Further research has demonstrated that the use of different analogues of PG (Estrumate, Synchrocept B and Lutalyse) in donor cows did not change the estrus response (30). Ovulation rate, embryo recovery or pregnancy rate per donor were not different when donors were treated with PG either 2 days or 3 days after gonadotrophin injection (31).

In Europe, the most common method of superovulation involves a single dose of PMSG (2000-2500 IU) during the mid-luteal phase of the estrous cycle followed 48-72 hours later by a luteolytic dose of $PGF_{2}\alpha$ (30 mg) or its analogue (cloprostenol) in the range 0.5-1.0 mg (32). As a result of the long half life of PMSG and its associated continuation of follicle stimulation, or because of rescue of atretic follicles which fail to ovulate (9), the plasma concentrations of estradiol in donors remain high into the luteal phase after the superovulation (33). These elevated concentrations of estradiol may be responsible for suboptimal survival rates of embryos following transfer to recipients(34).

PMSG has the advantages of being available in large quantities and at relatively low cost as compared to the gonadotrophins of pituitary origin (FSH-P). Its use also reduces the labour cost because only single injection is required to superovulate a cow. Other factors such as restricted supply of other agents (FSH-P) made its use more common in certain parts of the world (Europe and Canada). Since PMSG is not often available in the United States of America, FSH is more commonly used to induce superovulation in cattle. Futhermore, due to poor access to beef cows, especially when on pasture, the use of PMSG has been more widely used for these animals. In dairy cows, on the other hand, indoor management facilitates the use of multiple injections required to superovulate using FSH-P.

Hormone changes during superovulation with PMSG

Figure 1 represents the endocrine changes occurring in cattle superovulated with PMSG. This schematic diagram was drawn based on the information available in the literature. However, wide variation in trial design necessitate caution when interpreting specific values of hormone concentration.

(i) Progesterone:

The administration of PMSG initially exerts a transient luteotrophic effect in cows (17,28,35). This effect has been related to the LH-like activity of PMSG (17,20). The rise in progesterone concentrations may vary from 22%-158% (33) and can be detected 24h after the treatment with PMSG (27). Forty-eight hours after PMSG administration, progesterone concentrations in plasma had almost doubled (17). Fortunately, these levels can be induced to decrease to < 1 ng/ml in a period of 10-32h by the administration of PG (27,33). In the majority of donors, the concentrations of progesterone are low at estrus (< 1 ng/ml). In certain animals, however, progesterone concentration remains elevated at estrus (27,36). It has been suggested that this occurs in such animals because of either an inadequate dose of PG to induce complete luteolysis due to the luteotrophic effect of PMSG (37) or because of progesterone secretion by luteinization of large follicles, present at the time of treatment (38). Either of these could result in high progesterone concentration at the time of estrus (36). Also, premature ovulation has been observed in PMSG-treated cows (28). The luteal tissue resulting from premature ovulations would not be susceptible to PG treatment and it may produce enough progesterone to inhibit further ovulations (18).

Two days after estrus, progesterone concentrations start increasing and reach maximum concentration on day 14 (38,39). Following estrus, progesterone levels increase more rapidly in animals with higher ovulation rates and maximum concentration vary widely (27,38,39). Greve and his coworkers compared the hormone profile (progesterone and LH) in dairy cows superovulated with PMSG and FSH-P and reported that following estrus the yield of embryos and ova is not affected by the increasing rate of progesterone concentrations (27).

(ii) Gonadotrophins:

The effect of PMSG on endogenous production of gonadotrophins is controversial. A number of studies indicate no effect of PMSG on endogenous LH output (16,27,35,40). In contrast, however, a significant increase in endogenous LH has been reported in young animals (39). More studies are required, therefore, to investigate the effect of PMSG on the production and release of the animal's own gonadotrophins. The preovulatory surge of LH occurred 41h after PG treatment in animals superovulated with PMSG and at 65h in animals non-superovulated (36). In several other studies, approximately similar timings of the LH surge in animals superovulated with PMSG have been reported (16,27,35,40). The LH surge occurred earlier in PMSG treated cows than in cows treated with FSH or not superovulated (27,35).

(iii) Estradiol-17β:

In PMSG-treated cows, plasma concentrations of estradiol start increasing 24h after treatment and reach maximum concentrations between 36-52h after PG injection (33). At estrus, the concentrations of estrogens may be four times higher in superovulated animals than those of untreated animals (35,38,41,42,43). The decline in concentrations of estrogens after estrus is followed by a secondary rise around day 5 and 6, which may be eight times higher than the concentrations found at this stage in normally cycling animals. This secondary rise in estrogen concentrations may persist until day 18 (estrus=day 0) (38). Presumably this estradiol is related to the new follicular growth induced by a persistent effect of PMSG (33).

B. Follicle Stimulating Hormone (FSH):

Porcine pituitaries are commonly used for FSH preparations. FSH extracted from pituitary homogenates is lyophilized to prevent its damage under normal storage conditions. The short half life (approximately 5h) of exogenous FSH in cattle necessitates either frequent injections or other means of maintaining sufficient concentrations of this hormone to induce superovulation in cows (17,44). In order to maximize superovulatory response, doses of FSH ranging from 32-50 mg have been used either as one or two injections per day. Various treatment regimes have been studied. A wide range of treatment schedules are reported in literature including decreasing constant or increasing doses administered over a 4-day or a 5-day period (31,45,46,47,48,49,50,51,52).

Attempts to extend the period of absorption from the injection site using cellulose or gelatin does not reduce the requirement from 2 injections to one per day (53,54,55). In fact, use of such agents increased the variability in ovulation rate (55,56). High rates of superovulation can be obtained without any additives (48,54). In a recent study, Chupin and Procureur (53,54,55) concluded that neither increasing the dose of FSH from 32-50 mg nor the volume of saline used with each injection significantly affected ovulation rate, recovery of embryos and proportion of good embryos collected. They recommended that an optimal superovulatory treatment with FSH would require a total dose of 32 mg for cows or 24 mg for heifers, given twice daily for four days in decreasing doses with PG administered 48h after the first FSH injection. They concluded that an exact rate of decrease of FSH dose was not critical.

The removal of LH contamination from commercial FSH preparations increases the responsiveness of the cow to FSH (13,57). Although the amount of FSH in commercial preparations does not vary significantly between batches, there is a wide variation in the LH content. The ratio of FSH:LH is crucial; 10:1 ratio gives optimum superovulatory response (51). In Friesian cows, increased amounts of LH contaminating FSH preparations decreased ovulation rate (51). In Charolais, however, an increase in LH component of FSH increased both the number of ovulations and the number of transferable embryos (58). There is a pressing need to develop sources of pure FSH and LH separately, so that the optimal ratio of FSH:LH can be determined for different breeds. Like PMSG, commercially available crude FSH preparations contain both FSH and LH activities. A wide variation in the FSH/LH ratios ranging from 1-0.5 was detected in two batches (562C82 and 558H81, Burns-Biotec) of FSH (51).

There are reports which indicate that the embryo recovery and pregnancy rates per donor were higher in animals superovulated with FSH than those treated with PMSG (15,31). The use of FSH has been particularly beneficial in dairy cows since good ovulation and fertilization rates were obtained even when cows were superovulated at their peak of lactation. Milk production was not affected. These benefits probably outweigh the problems of multiple injections of FSH and, consequently, its use is more common in North America.

Hormone changes during superovulation with FSH-P:

Unlike PMSG, very few studies have been conducted which describe the endocrine changes in FSH-P superovulated donors. Information available in the literature on these changes is presented in Figure 4.

(i) Progesterone:

Treatment with FSH also exerts a luteotrophic effect. The increase in progesterone concentrations in FSH-P-treated cows were similar to that found in PMSG-treated animals (28). In contrast, however, other researchers did not observe a stimulatory effect FSH-P on corpus luteum function in Holstein cows (49). The FSH (FSH-P), Burns Biotec, Oakland, CA) used in this experiment has less LH contamination and a progesterone increase was not

observed following the treatment. Following treatment with $PGF_2\alpha$, the concentrations of progesterone declined precipitously and were below 1 ng/ml within 12h. Consequently, estrus occurred earlier in FSH-P treated animals (52.8h and 42h for total dose of 32 mg and 50 mg, respectively) than in controls (73.5h). At the time of estrus, the concentrations of progesterone were below 1 ng/ml in all the animals. In superovulated animals, the concentrations of progesterone again increased 78h after estrus, however the rise in progesterone concentration was not observed until 107h in animals which were not superovulated.

(ii) Gonadotrophins:

As has been demonstrated in PMSG-treated animals, endogenous production of the gonadotrophins was increased following treatment with FSH-P. A significant increase in plasma concentrations of both FSH and LH has been found for approximately 36h after the first injection of FSH-P (49). These elevated levels returned to basal concentrations before the last FSH-P injection. It was hypothesized that stimulation of either the hypothalamus or the anterior pituitary resulted in increased secretion of gonadotrophins in superovulated animals.

It has been demonstrated that the preovulatory surge of FSH and LH in animals treated with FSH-P occur together at a precise time (59). However, the exact timing of the surge and its relationship to the occurrence of behavioural estrus are dependent upon factors such as dose, age, breed and season. The results of one comparative study showed that the onset of the LH surge occurred at 40h and 44h after the treatment with the cloprostenol in PMSG and FSH-treated cows, respectively (28).

(iii) Estradiol-17β:

Experimental results have indicated that plasma concentrations of estradiol-17β did not change until 48h after treatment with FSH-P. After this time, concentrations increased rapidly and reached a maximum (42 ± 8 pg/ml) at 46 ± 3h following the treatment with $PGF_2\alpha$ (49). In another study, concentrations of estradiol-17β prior to the onset of estrus were 2-4 times higher than those of animals which were not superovulated (28). After the estradiol peak, estrus occurred within 2h and FSH and LH surges within 3 hours. The concentrations of estradiol decline rapidly at the time of the LH peak and remain low until the days of embryo collection (59).

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C. Human Menopausal Gonadotrophin (hMG):

hMG is extracted from post-menopausal human urine. It is supplied as a lyophilized powder in vials containing 75 IU of FSH and 75 IU of LH (60). It has been demonstrated that hMG preparations differ with regard to FSH:LH ratio (13) and its clinical activity is similar to that of human pituitary gonadotrophin (60). Various reports have demonstrated that hMG can be used effectively to superovulate cattle for transfer of viable embryos (14,61,62). The higher cost of this product, and the lack of a substantial advantage over FSH-p will limit its use in the embryo transfer industry.

Onset of Estrus in Superovulated Animals

After the administration of PG, it has been demonstrated that estrus occurs on the second day in superovulated animals as compared to the third day in animals which were not superovulated (36,49). Comparing the effect of two superovulatory regimes (PMSG and FSH-P) researchers have shown that, in Friesian cows, estrus occurred at 46h and 52h after PG in animals superovulated with PMSG and FSH, respectively (28). In other experiments, estrus occurred 59 ± 5h after PG in beef heifers (59) and 44.2 ± 1.4h after PG in dairy heifers (63).

In superovulated animals, signs of estrus may occur at varying times in relation to the preovulatory LH peak. Demonstrated heat has occurred before the preovulatory LH peak, at the time of the LH peak or after the LH peak (28,35,49,59). Superovulated animals have a more rapid decline in plasma progesterone concentrations after PG treatment and an earlier rise in estrogen concentrations. This may contribute to a more rapid onset of the LH peak and estrus (35,49).

In some animals, superovulation with PMSG results in higher concentrations of progestereone at the time of estrus than in controls. High progesterone concentrations at this time may either inhibit the release of LH or completely block the surge of LH (28,36). The inhibitory effect of progesterone on estradiol induced LH release has been well demonstrated (64,65). Elevated progesterone concentrations at the time of insemination have also been shown to reduce fertilization rates in superovulated cows (66). The deficient or reduced LH production may adversely affect oocyte maturation, ovulation and subsequent fertilization (28,67,68). Irrespective of the superovulatory regime, a large proportion (20-34%) of donors do not show signs of estrus (19,69,70,71).

Prediction of superovulatory response:

Plasma concentrations of progesterone at the time superovulation is initiated does not influence ovulation rate, recovery of embryos or their quality (28,72). Superovulatory treatments performed during the mid-luteal phase, when progesterone concentrations are high, result in increased ovulation rates. However, this increase was related to stage of the cycle rather than to progesterone concentrations (63,72). Attempts to increase the number of ovulations by the administration of exogenous progesterone from day 3 to 10 of the estrous cycle did not succeed (21).

Measuring plasma or milk levels of hormones such as progesterone, PMSG, LH or estrogen has not been shown to be useful in predicting the number of ovulations (33,40,41,42,72,73). Hormonal concentrations often indicate whether an animal has responded to superovulatory treatments but cannot be used to predict the number of ovulations. The administration of hCG or GnRH at estrus in superovulated cows did not reduce the number of unovulated follicles and had no influence on ovulation rate (74,75,76,77).

Effect of superovulation on follicular population

Very few studies have been conducted to assess the follicular changes which occur after stimulation with PMSG or FSH in cattle.

Superovulation with PMSG has been shown to either prevent normal follicles from becoming atretic or rescue some early atretic follicles from atresia (9). It is proposed that some large follicles rescued from atresia may ovulate but, more commonly, these follicle luteinize following the preovulatory surge of LH (9). Unfortunately, no studies have been conducted to evaluate the quality of oocytes from follicles which were in the early stages of atresia when rescued by PMSG. There is a need to investigate the causes of degeneration of oocytes and embryos recovered from superovulated cows. It has been demonstrated that premature activation of the oocyte during superovulation is an important source of abnormal embryos (78). Some of these oocytes are retained in luteinized follicles, while others are ovulated as aged eggs. Whether degenerating embryos originate from aging of oocytes or from imperfect oocytes rescued from early atretic follicles is not known.

One of the long term effects of superovulation with PMSG is the induction of large, unovulated follicles (> 10 mm diameter) detected at the time of embryo recovery or laparotomy of donors around day 7 after insemination (17,72,79). Large unovulated follicles (> 10 mm diameter) have been found in varying numbers in ovaries superovulated with PMSG (72,75,80). These unovulated follicles are those which apparently continued to grow due to the continuous stimulatory effect of the FSH-activity in PMSG but could not ovulate because of the sharp increase in progesterone secretion after the surge of LH (17).

Superovulation with PMSG on different days of the estrous cycle does not effect the presence or number of non-ovulated follicles found at the time of embryo recovery (78,81). Although there is no significant correlation between the total number of corpora lutea and the total number of unovulated follicles (33,38), the number of unovulated follicles was negatively correlated with the time interval between PMSG administration and onset of estrus (72).

The administration of anti-PMSG serum at estrus significantly reduced the number of unovulated follicles (82). Anti-PMSG has become commercially available. Its use may improve embryo recovery, reduce excessive post-ovulatory follicle growth and enhance the application of PMSG as a superovulatory drug for cows.

If cows are superovulated repeatedly with PMSG, a temporary decline in ovulation rate occurred following the first superovulation (83,84). It has been suggested that this decline in ovarian response may result from temporary depletion of the class of follicles which can be stimulated by PMSG (85). Since the action of PMSG is persistent, the use of anti-PMSG should minimize this period of ovarian insensitivity.

Researchers have demonstrated that either atretic or luteinized follicles are capable of producing significant amounts of progesterone which may further contribute to already high concentration of this hormone secreted from multiple corpora lutea. As a consequence, estrous cycles are often extended in superovulated cattle (38,39).

After ovulation, due to the long biological half life of PMSG, small follicles are stimulated sufficiently to grow and secrete large amounts of estradiol which can be detected 3 days after estrus (33). High estradiol concentrations at this time may accelerate the transport of ova through the oviducts and lead to premature shedding of the zona pellucida and subsequent degeneration of the eggs. One advantage of using FSH as superovulatory drug in cattle is the reduced incidence of prolonged follicular stimulation (54).

Synchrony of donors with recipients:

Survival of the transferred embryo depends on a close alignment between the developmental stage of the embryo and the stage of the uterus of the recipient. Synchronization is usually ensured by using the onset of estrus as reference point in matching the donor and recipient animals.

Maximal pregnancy rates (91%) have been achieved when recipients and donors are exactly synchronized at the onset of estrus (32). Recipients out of phase by ± 1 days had lower pregnancy rates (52-56%) (86). However, other researchers have reported little gain in pregnancy rates when estrus in recipients and donors was aligned exactly (63%) compared to when recipients were one day ahead (-1) of donors (60%) (89). In another study, pregnancy rates in recipients which exhibited signs of estrus 12h ahead of donors and those in which estrus was synchronized exactly with donors were similar (90). In both of these reports, lower rates of pregnancy were recorded when recipients exhibited estrus 12-24h later than the donors (89,90).

It is not known why accurate synchrony between donors and recipients is necessary for a successful pregnancy. In cattle, the administration of progesterone or hCG for 20 days after insemination has been shown to be effective in reducing early embryonic loss in problem herds (91,92). While progesterone probably plays an important role in

influencing uterine condition in recipients, estrous synchronization is the only method for ensuring good pregnancy rate in intact animals (18).

Recently, it has been demonstrated that recipent uterine environment at transfer is of greater importance than the origin of the embryos (93). Embryos from repeat breeder donors, when transferred into virgin heifers resulted in acceptable pregnancy rates. Embryos transferred to heifer recipients resulted in higher pregnancy rates than those transferred to repeat breeder recipients.

While synchrony of estrus between recipients and donors is necessary to obtain optimal pregnancy rates, in practical situations however, this may be difficult to achieve because some donors and recipients do not exhibit estrus (17,70). Since the period between the preovulatory LH surge and the time of ovulation is constant (94), a more precise alignment of recipients and donors would be expected if the time of the LH surge or ovulation could be used as a reference. If a rapid assay system for LH was available it could be used effectively in matching the recipients and donors using the LH surge as a reference point instead of estrus signs. In a recent study using superovulated beef heifers, the time between LH surge and suggested insemination was ____ hours (95).

Ovulation time and insemination of donors:

In order to determine the appropriate time for insemination of donor cows two methods are commonly used. Either cows are carefully observed for onset of estrus or inseminations are done at fixed times after $PGF_2\alpha$ administration. The traditional approach has involved multiple inseminations (3-4 times) at 12h intervals during and immediately after estrus to ensure good fertilization rates (96,97). These methods have been based on the assumption that ovulations start at a precise time after the onset of estrus and that ovulation occurs over 24-48h (98). However, a fairly large proportion of donors ovulate without demonstrating signs of estrus (70,71). In addition, the onset of estrus does not always predict the time of LH peak and ovulation (14,27). Insemination of donors at predetermined times after PG injection may prove to be more reliable than breeding on the basis of estrus alone. Other than the benefits of not having to observe for signs of estrus, if donors were inseminated at predetermined times, fertilized embryos would be expected from cows which ovulated without demonstrating signs of estrus.

It was not known, until recently, whether ovulations in superovulated animals occurs simultaneously in all the follicles which are stimulated to grow, or whether ovulations will be extended over a number of hours. Laparoscopic studies have suggested that in cows superovulated with PMSG, 45% and 91% of ovulations occurred within a period of 24 and 48 hours after the onset of estrus, respectively. No ovulations were observed in the first 18 hours (99,100). It must be noted that laparoscopic studies have definite weaknesses of viewpoint and timing of observations. Recent reports suggest that ovulations in superovulated cows may occur over a short time period (100,101). The conclusions drawn were that a single insemination with two units of high quality semen, 24h after the onset of estrus, should result in optimal conception rates. Results were considerably less when insemination was 12h after the onset of estrus.

A recent study has demonstrated that in beef heifers, superovulated with FSH, ovulations occurred over a relatively short period of time (94). The results of this experiment suggests that one insemination at 72 hours following the onset of estrus would be properly timed for all ovulations. The experimental methods used involved slaughtering the heifers at known times after PG treatment in order to actually visualize an ovulation pattern (94).

Although there are no large differences in fertilization rates obtained using fresh or frozen semen, one insemination with fresh semen has been demonstrated to be sufficient (17). Spermatozoa which have not been frozen and thawed may survive longer in the genital tract of the cow (32). It has also been suggested that fertilization rate in donor cattle inseminated with frozen semen may be markedly influenced by individual bulls (98).

Ovulation, fertilization, recovery rates and quality of embryos

Attempts to induce predictable numbers of ovulations in cattle have been unsuccessful. Number of ovulations in response to available gonadotrophin preparations are still notoriously variable, even when donors are given the same superovulatory treatment (1). In cows induced to superovulate with PMSG (2000 I.U.) a wide range (3-70) in number of ovulations have been reported (102). Factors such as dose and batch of gonadotrophin, breed, stage of estrous cycle, nutrition, season and genetic variation between animals have all been suggested as prime causes of the variation in ovulation rate. Conversely, it has been argued that there may be little difference between a dose of gonadotrophin which stimulates follicular development and one which is ineffective (103).

Evaluation of the superovulatory response by rectal palpation has been widely used. This is generally inaccurate and often gross underestimates are recorded when the number of corpora lutea on any one ovary exceeds nine (75). This probably results in an overestimation of embryo recovery. Similarly, oocyte cleavage is generally used to determine the fertilization rates. Fertilization rates would, however, be expected to be higher than the cleavage rate. Cleavage rate after fertilization of oocytes in the bovine is unknown. No objective tests are available to evaluate the quality of an embryo. The evaluation employed is subjective and may vary from one observer to another.

Multiple insemination of donors around the time of estrus has been a common practice to ensure optimal fertilization rate (17). Researchers have recovered a fairly large number of unfertilized ova (19.5% in cows and 7% in heifers) which they thought was because of prolonged ovulation (104). It would be expected that by the time last ova arrives in the oviducts, fertilization may not occur because the spermatozoa have lost their ability to fertilize. Unfertilized oocytes are often collected at flushing, either as part of an otherwise normal collection of embryos or as the total collection. Both total and partial fertilization failure are common in embryo transfer programs. However, fertilization rates as high as 87% and 90% have been obtained in FSH superovulated beef cows (101).

Fertilization failure may, however, not limit success as severely as failure to recover all the embryos. It is estimated that only 50-80% are recovered (104). Both fertilization and embryo recovery rates tend to decrease as ovulation rates increase. Loss of embryos may result from early embryonic death, lysis, or loss through the vagina due to embryo-uterine asynchrony, or from failure of follicles to shed their oocytes. As a general rule, however, the number of recovered embryos still increases with ovulation rate (104) and the number of transferable embryos per collection correlates with total embryos and ova in a collection (105). Other workers are of the view that the fimbria may not pick up all the oocytes because the ovaries are grossly enlarged (1).

Repeated superovulation

In several reports, a decrease in number of ovulations and embryos was reported following repeated superovulation with PMSG (15,84,106,107,108,109,110). The reduction in ovulation response was thought to be because of formation of antibodies against PMSG which react with PMSG administered during subsequent superovulation treatments (84,108). However, antibodies against PMSG were not detected (84). Other researchers did not observe a reduction in ovulation rate, even after 5-7 superovulations with PMSG. Again, antibodies against PMSG were not detected (17). A proposed explanation was that exhaustion of available follicles which could be stimulated by PSMG or interactions in gonadotrophin feedback mechanism may have been responsible for decrease in ovulation rates (17).

Although the number of follicles in the ovaries of a cow are very low after 15 years of age (111), ovarian function seems to continue until all follicles disappear (112). Animals which showed a poor ovulation rate to first treatment of PMSG continued to show poor ovulatory response in successive superovulations (84). The total number of normal follicles in the ovaries before and after superovulation are more variable than the follicles between ovaries of an animal (80). Perhaps, a critical mass of growing follicle is a prerequisite for the maintenance of normal folliculogenesis and response to superovulation (113).

Conclusions

Since the development of modern hormone assay techniques the knowledge base related to our understanding of reproductive endocrinology has expanded precipitously. It has been the objective of this two part series on bovine endocrinology to consolidate many of the detailed observations that have been made on the complicated, intricate hormonal relationships involved in the bovine estrous cycle and superovulation. It is only through our adequate undestanding of these events and changes that new reproductive techniques will be developed and utilized.

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