

Physiological aspects of reproduction and fertility in dairy cows

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Abstract

Reproductive efficiency is an important aspect of dairy cattle production. To improve it in high yielding dairy cattle, a thorough understanding of the physiology of fertility and the reproductive physiological implications of the increasing metabolic demands associated with increasing production levels is essential. In this respect, the present paper focuses on the neuro-endocrine regulation of ovarian activity, physiological aspects of fertility in the postpartum cow and physiological aspects of regular and irregular return to oestrus after insemination.

1. Introduction

An efficient reproduction in dairy cattle herds is of great economic importance. With the genetic improvement of dairy cattle for milk yield, demands for nutrients have largely increased, especially in early lactation. In situations in which these demands can not be met by feed intake, body reserves will be reallocated for production, causing physiological and even pathophysiological changes which affects reproduction and health. In this paper health will not be considered. The objective is to provide general background information about physiological aspects of fertility in dairy cows and to indicate pathways through which increased demands for production might interfere with reproduction.

2. Neuro-endocrine regulation of ovarian activity

In dairy cattle, as in all mammalian species, ovarian activity is regulated by the pituitary hormones LH (luteinizing hormone) and FSH (follicle stimulating hormone). The secretion of these two gonadotrophins, in turn, is primarily regulated by the secretion of GnRH (gonadotrophin releasing hormone) from the hypothalamus. GnRH is secreted in a pulsatile manner and it is assumed that the GnRH pulse generator is located within the hypothalamus. Electrophysiological studies are now in progress (especially in the goat as a model) to

improve our understanding of the mechanisms through which various factors modify the electrical activity of the GnRH pulse generator (Mori and Tanaka, 1995). Various neuro-endocrine stimuli are known to affect the GnRH pulse generator, but the actual electrophysiological mechanisms involved are still unclear. The pulsatile secretion of GnRH is not only influenced by internal stimuli and feedback mechanisms, but can also be influenced by environmental factors such as season, nutrition and social interactions, explaining at least some of the effects of these factors on reproduction.

2.1 Ovarian activity at puberty

Long before puberty, the pituitary can already respond to GnRH, but the frequency of release of GnRH from the hypothalamus is low. As the hypothalamus mature during the prepubertal period, the pulse frequency increases. As a consequence the LH pulse frequency also increases and ultimately reaches the level that is needed to enhance the development of ovarian follicles. Once these follicles are able to develop to the stage in which they produce large amounts of oestrogens, behavioral oestrus and a preovulatory LH surge will be induced, the latter leading to the first ovulation (Kinder et al., 1995). After this, as long as the cow remains non-pregnant, ovulation will occur with regular intervals of 18-24 days.

2.2 Regulation of the oestrous cycle

From a regulatory point of view the preovulatory LH surge is the pivotal step in the oestrous cycle because it induces ovulation. During the period preceding ovulation, the follicular phase of the cycle, a large follicle emerges from a pool of developing small follicles. This follicle, referred to as the dominant follicle, is destined to ovulate if a LH surge occur at the appropriate time. The oestrogens produced by this follicle induces a neuro-endocrine signal which, in turn, induces a large surge in the secretion of GnRH (Caraty et al., 1995). Thus, through a positive feedback mechanism of oestrogens on the release of GnRH, the dominant follicle induces the preovulatory LH surge. Apart from its effect on the hypothalamus, the oestrogens produced by the dominant follicle also increases the sensitivity of the pituitary for GnRH.

After ovulation, the remnants of the dominant follicle will luteinize to become the corpus luteum. Whereas the follicle mainly produces oestrogens, the corpus luteum produces progesterone. After a period of progesterone priming, uterine tissue, more specifically the endometrium, starts to produce low amplitude pulses of PGF2a. This PGF2a has a negative effect on the corpus luteum function, initiating the process of luteal regression. In cyclic animals ultimately a large pulse of PGF2a develops and causes the final stages of regression of the corpus luteum (Thatcher et al., 1995). It is this luteolytic mechanism that controls the length of the luteal phase of the cycle. With the decrease in circulating progesterone levels after luteal regression, a new dominant follicle emerges from the pool of developing small follicles and a next cycle commences.

The regulation of the uterine PGF2a production leading to regression of the corpus luteum involves ovarian oxytocin (actually oxytocin produced by the corpus luteum itself) and endometrial oxytocin receptors (OTR). Through a positive feedback loop ovarian oxytocin pulses stimulate the pulsatile release of endometrial PGF2a which, in turn, stimulates the release of ovarian oxytocin (Heap et al., 1986).

2.3 Ovarian activity in the postpartum period

During pregnancy the ovarian activity is low, mainly because the level of circulating LH is very low. Follicles with a diameter of more than 5 mm are rarely seen at the time of parturition. During early lactation the LH concentration in blood starts to increase. This increase is due to a higher LH pulse frequency rather than a higher amplitude of the individual LH pulses. It is this increased pulse frequency which is essential for re-initiation of ovarian activity, resulting in the first postpartum ovulation and subsequent commencement of luteal activity (i.e. the first postpartum rise of the level of circulating progesterone). The first postpartum ovulation is often not accompanied by overt oestrous behaviour. The only reliable way to detect it is therefore progesterone measurement, either in blood or in milk.

The literature on postpartum patterns of growth of ovarian follicles have recently been reviewed by Jolly et al. (1995). Although follicles with a diameter of more than 10 mm can be seen in almost all cows within 10 days after calving, the first dominant follicle to occur will not ovulate in cows which are underfed. In fact, before the first ovulation, 1 up to 9 dominant follicles with progressively increasing maximum diameter may develop and regress in underfed cows. The available data show that the maximum size and the total lifespan of the second dominant follicle after calving are inversely related to the interval from calving to first ovulation.

The length of the luteal phase following the first postpartum ovulation seems to depend on the time of first postpartum ovulation. Savio et al. (1990) found normal (18-24 days) or long (> 25 days) first ovarian cycles in cows in which the ovulatory dominant follicle was detected before Day 10 postpartum. For cows in which the ovulatory dominant follicle was detected after Day 20 postpartum, the duration of the first ovarian cycle was consistently short (9-13 days). Within the intermediate group of cows, short normal and long first ovarian cycles were found. A possible explanation for this is the degree of progesterone mediated preconditioning of the uterus and its effect on the luteolytic mechanism. In cows in which the first dominant follicle develop very early after

calving, the uterus is still conditioned by the high circulating progesterone levels of late pregnancy. In contrast, in cows in which the first dominant follicle develops relatively late, the uterus has not been under recent progesterone dominance. Both cases may result in a dysfunction of the luteolytic mechanism, leading to a prolonged or a shortened luteal phase, respectively (Savio et al., 1990; Lamming & Mann, 1995).

3. Physiological aspects of fertility in the postpartum cow

3.1. Postpartum interval to first ovulation

Despite the fact that almost all cows have their first ovulation followed by commencement of luteal activity before Day 50 of lactation, the time of onset of ovarian activity is important for the chance to get pregnant after first insemination in the period in which cows are normally inseminated (Day 60 onwards). This has already been reported in the early seventies for cows which were serviced for the first time after Day 60 postpartum. Cows which had 0 (25.9%), 1 (38.7%), 2 (28.0%), 3 (7.2%) or 4 (0.3%) heats before Day 60 postpartum, required 2.60, 2.58, 2.32, 2.21 and 1.75 services to become pregnant, respectively (Thatcher and Wilcox, 1973). These results were recently confirmed by Darwash et al. (1997), using data already collected between 1975 and 1982 (!). In their analyses, these authors included effects of herd, year, season and parity. For cows with an interval to postpartum commencement of first luteal activity of <12 (1.7%), 13-24 (50.3%), 25-36 (32.3%), 37-48 (10.6%) and >49 (5.1%) days, the days to conception was 71.7, 84.7, 89.6, 91.6 and 97.2, respectively. The mean number of services required for conception was 1.28, 1.49, 1.50, 1.58 and 1.64, respectively. Apart from the studies of Thatcher and Wilcox (1973) and Darwash et al. (1997), several other studies has indicated the same phenomenon (Staples et al., 1990; Lucy et al., 1992; Senatore et al., 1996).

The mechanism underlying the effect of number of cycles before insemination on the success of insemination in terms of sustained pregnancy is not known. It may be related to the quality of the environment in the female

reproductive tract. It is possible that repeated hormonal conditioning of the oviductal and/or uterine tissues, i.e. the subsequent uterine exposure to periods of oestrogen dominance and progesterone dominance, has a favourable effect on the events leading to fertilization and/or on the intra-uterine environment, essential for embryo survival. However, it can also be related to oocyte quality. It can not be excluded that the quality of the dominant follicle, and thus the oocyte, increases with an increasing number of postpartal follicular growth waves. Both possibilities deserve further investigation.

Since the length of the postpartum interval to first ovulation (i.e. commencement of luteal activity) affects fertility at the time that dairy cattle are normally inseminated, it is of interest to know the factors which influences this interval. Two factors which are of interest in this respect are (1) the interaction between normal postpartal PGF2a release and the occurrence of bacterial contamination of the uterus during parturition and (2) the energy balance during early pregnancy.

Postpartal PGF2a release in relation to bacterial contamination of the uterus during parturition. In cows, parturition is associated with a high incidence of bacterial contamination of the uterus. As a consequence, the normally occurring postpartal release of PGF2a is largely increased in comparison to non-contaminated cows. By comparing cows with a totally uninfected uterus with cows with a varying degree of uterine infection, Fredriksson et al. (1985) found that uninfected cows ovulated earlier (16.3 ± 5.2 days) than infected cows (30.5 ± 12.9 days). Whereas in the uninfected cows no relationship was found between the duration of postpartal PGF2a release and postpartum interval to first ovulation (Fredriksson et al., 1985), Madej et al. (1984) found a negative correlation between the duration of postpartal PGF2a release and postpartum interval to first ovulation followed by a luteal phase of normal length (thus not including short first oestrous cycles). In cows with bacterial contamination of the uterus, Fredriksson et al. (1985) found an increased postpartum interval to first ovulation with increasing duration of PGF2a release, or in other words, a positive correlation.

The release of PGF2a during the postpartum period seems to have a role in uterine involution in a sense that a certain amount of PGF2a is necessary for the normal involution process. In cows without any bacterial contamination of the uterus during parturition, a longer duration of PGF2a release is related to a faster uterine involution. However, if a long duration of PGF2a release is a consequence of bacterial contamination of uterus, the involution process is retarded. Here not the duration as such, but the vast amounts of PGF2a, owing to the inflammatory process, is detrimental for uterine involution (Kindahl et al., 1992)

Postpartal energy balance. Early lactating cows have a slight to severe negative energy balance. It lasts for 4 to 12 weeks and is directly related to milk production. The relationship of the severity of this negative energy balance with the postpartum interval to first ovulation has been subject of many studies. These studies either indicated a positive relationship (Butler et al., 1981; Staples et al., 1990; Canfield and Butler, 1990; Canfield et al., 1990; Spicer et al., 1990; Canfield and Butler, 1991; Lucy et al., 1992; Whitaker et al., 1993; Senatore et al., 1996; Jonsson et al., 1997) or the absence of a relationship (Ducker et al., 1985; Villa-Godoy et al., 1988; Harrison et al., 1990). Several studies (reviewed by Jolly et al., 1995) have shown that the LH pulse frequency is suppressed in cows which have a higher nutrient demand than nutrient intake. As already indicated above, high frequency pulsatile release of LH is essential for the re-initiation of ovarian activity. Thus prolonged postpartum intervals of ovarian inactivity seems to be related to the effect of a negative energy balance on the GnRH pulse generator. Although research will have to confirm this, it is likely that the effect of a negative energy balance on the GnRH pulse generator in cattle is mediated by leptin (Yu et al., 1997) and/or neuro-peptide Y (Kuenzel and Fraley, 1995). Leptin, secreted by adipocytes, is known to augment the release of GnRH from the hypothalamus and FSH and LH from the pituitary. It may also augment the response of the pituitary to GnRH. Neuropeptide Y has drawn much attention because it stimulates food intake considerably. It also augments

GnRH's capacity to effect LH release from the pituitary.

3.2. Postpartum effects on follicle and oocyte quality

From the total pool of primordial follicles in the ovaries, regularly a small group starts to develop to the antral stage. The time needed for development to the early antral stage is not exactly known for the cow, but based on work in other species it is assumed to be at least 40 days (Britt, 1993). The development of a follicle from the early antral stage to the late preovulatory stage in the cow has been investigated and requires approximately 40 days (Lusier et al., 1987). This means that any follicle that ovulates in the period around Day 80 postpartum, was in the early preantral stage of development during the first few weeks of lactation. Note that this is independent of the fact whether this follicle is the first, second, third or even fourth to ovulate postpartum! Since this is the period in which lactating dairy cows are in a slight to severe negative energy balance, it was postulated by Britt in 1991 (cited by Britt, 1993) that the quality of the follicle ovulating by Day 60 to 80 postpartum may be poorer than that of follicles ovulating earlier or later, especially when the negative energy balance is severe. As a consequence, the oocyte quality and/or the quality of the corpus luteum developing from this follicle after ovulation may be suboptimal.

Some evidence for effects of postpartum energy balance on oocyte quality comes from an in vitro study Kruijff et al. (1996). The negative energy balance during early lactation is accompanied by lipolysis. As a consequence, the amount of non-esterified fatty acids (NEFA) in the blood increases. Especially with a severe negative energy balance, the high levels of NEFA may cause the accumulation of tri-acyl-glycerol (TAG) in the liver, resulting in a fatty liver. By using in vitro maturation and in vitro fertilization (IVM/IVF), Kruijff et al. (1996) have shown that oocytes recovered from the ovaries of cows which developed fatty livers have a reduced developmental capacity, especially when collected between Days 80 and 120 of lactation. The oocytes collected were all aspirated from follicles with a diameter of more than 2 mm. The criterium for

developmental capacity of the oocytes was their ability to develop during 9 days after IVF to at least the early blastocyst stage with more than 30 cells.

4. Physiological aspects of regular and irregular return to oestrus after insemination

In cattle luteal progesterone is essential for maintenance of pregnancy. After successful insemination the embryo itself prevents regression of the corpus luteum by producing an anti-luteolytic protein, interferon tau (IFNT), initially referred to as bovine trophoblast protein-1 (bTP-1). This maintenance of corpus luteum function is associated with an absence of pulsatile PGF2a secretion by the uterine endometrium. The complete process through which the embryo rescues the corpus luteum from the luteolytic mechanism that operates during the oestrous cycle is referred to as maternal recognition of pregnancy. In cattle, maternal recognition of pregnancy occurs at Day 16-17 of the cycle.

The antiluteolytic effect of IFNT is almost certainly limited to the uterine lumen. Until now there is no evidence that IFNT is released into either the uterine venous or lymphatic drainage, excluding systemic effects. Through its effects on the expression of, amongst others, the oestrogen receptor (ESR) and oxytocin receptor (OTR) gene, IFNT affects the endometrial production of PGF2a and thus interferes with the luteolytic mechanism of the oestrous cycle (for detailed reviews, see Geisert et al., 1994; Thatcher et al., 1995; Spencer et al., 1996).

Regular return to oestrus. If insemination does not lead to fertilization or if the fertilized oocyte does not develop into a viable embryo by Day 16-17 after insemination, the cow will return to oestrus after a cycle of normal length. In studies to investigate the effects of recombinant IFNT on PGF2a secretion, either in vivo or in vitro, it has been observed that approximately 20% of all cows does not respond to IFNT. Although more research is needed, this might indicate (temporary?) deficiencies in IFN receptors or post-receptor mechanisms (Thatcher et al., 1995). It should be realized that the inability of an early

pregnant cow to respond to the embryonic IFNT will lead to normal regression of the corpus luteum. As a consequence, the embryo will die soon after Day 16-17 and the cow will return to oestrus after a cycle of normal length. This will of course also be the case if the embryo fails to produce IFNT.

Irregular return to oestrus. Assuming that no inseminations takes place in cows which are not in oestrus, oestrous cycles of more than 24 days are the consequence of embryonic mortality after Day 16-17 after insemination. In these cows maternal recognition of pregnancy did occur, but as soon as the embryo dies the uterine endometrium resumes the synthesis of PGF2a, causing the delayed regression of the corpus luteum and resumption of ovarian cyclicity. Little is known about the time between death of the embryo (for other reasons than untimely luteal regression) and resumption of ovarian cyclicity in animals in which maternal recognition of pregnancy have already occurred (i.e. embryo mortality after Day 16-17 of pregnancy). Northway and French (1980) flushed embryos nonsurgically from the uterus shortly before or shortly after maternal recognition of pregnancy and found that cows flushed between Days 17 and 19 returned to oestrus between Days 25 and 27 after insemination. However, after natural death instead of flushing, embryonic remnants may still suppress the luteolytic mechanism for some time, resulting in resumption of ovarian activity after a longer interval. Evidence for this comes from an experiment in which embryos were killed intra-uterine (Kastelic and Ginther, 1989). In this experiment, ovulation occurred on average almost 21 days after embryonic death on Day 43 of pregnancy.

Based on the information available, the fertilization rate in cattle is about 90%, whereas calving rate after first insemination is 50 to 60%, indicating a high rate of prenatal loss. The majority of this prenatal loss is embryonic loss, i.e. loss before Day 42 of pregnancy. Based on the information available the majority of embryonic loss seems to occur before Day 18 of pregnancy (Sreenan and Diskin, 1986; Zavy, 1994). Taking this altogether it can be concluded that return to oestrus after a normal cycle is more often due

to embryonic mortality than fertilization failure.

References

- Britt, J.H. 1993. Impacts of early postpartum metabolism on follicular activity, cyclicity, fertility and embryonic death in cattle. Proceedings of the Symposium "Interaction of Nutrition and Reproduction" of the Dutch Council for Agricultural Research (NRLO), NRLO-report 93/17, 12pp.
- Butler, W.R., Everett, R.W., Coppock, C.E. 1981. The relationship between energy balance, milk production and ovulation in post partum Holstein cows. *J. Anim. Sci.* 53, 742-748.
- Butler, W.R., Smith, R.D. 1989. Interrelationships between energy balance and post partum reproductive function in dairy cattle. *J. Dairy Sci.* 72, 767-783.
- Canfield, R.W., Butler, W.R. 1990. Energy balance and pulsatile LH secretion in early post partum dairy cattle. *Dom. Anim. Endocr.* 7, 323-330.
- Canfield, R.W., Butler, W.R. 1991. Energy balance, first ovulation and the effects of naloxone on LH secretion in early post partum dairy cows. *J. Anim. Sci.* 69, 740-746.
- Canfield, R.W., Sniffen, C.J., Butler, W.R. 1990. Effects of excess degradable protein on post partum reproduction and energy balance in dairy cattle. *J. Dairy Sci.* 73, 2342-2349.
- Caraty, A., Evans, N.P., Fabre-Nys, C.J., Karsch, F.J. 1995. The preovulatory gonadotrophin-releasing hormone surge: a neuroendocrine signal for ovulation. *J. Reprod. Fert. suppl.* 49, 245-255.
- Darwash, A.O., Lamming, G.E., Woolliams, J.A. 1997. The phenotypic association between the interval to post-partum ovulation and traditional measures of fertility in dairy cattle. *Anim. Sci.* 65, 9-16.
- Ducker, M.J., Hagggett, R.A., Fisher, W.J., Morant, S.V., Bloomfield, G.A. 1985. Nutrition and reproductive performance of dairy cattle. 1. The effect of level of feeding in late pregnancy and around the time of insemination on the reproductive performance in first lactation dairy heifers. *Anim. Prod.* 41, 1-12.
- Fredriksson, G., Kindahl, H., Sandstedt, K., Edqvist, L.-E. 1985. Intrauterine bacterial findings and release of PGF_{2a} in the postpartum dairy cow. *Zbl. Vet. Med. A* 32, 368-380.
- Geisert, R.D., Short, E.C., Morgan, G.L. 1994. Establishment of pregnancy in domestic farm species. In: Zavy, M.T. and Geisert, R.D. (Eds.), *Embryonic Mortality in Domestic Species*. CRC Press, Boca Raton, pp. 23-51.
- Harrison, R.O., Ford, S.P., Young, J.W., Conley, A.J., Freeman, A.E. 1990. Increased milk production versus reproductive and energy status of high producing dairy cows. *J. Dairy Sci.* 73, 2749-2758.
- Heap, R.B., Rider, V., Wooding, F.B.P., Flint, A.P.F. 1986. Molecular and cellular signalling and embryo survival. In: Sreenan, J.M. and Diskin, M.G. (Eds.), *Embryonic Mortality in Farm Animals*. Martinus Nijhoff Publishers, Dordrecht, pp. 46-73.
- Jolly, P.D., McDougall, S., Fitzpatrick, L.A., Macmillan, K.L. & Entwistle, K.W. 1995. Physiological effects of undernutrition on postpartum anoestrus in cows. *J. Reprod. Fert. Suppl* 49, 477-492.
- Jonsson, N.N., McGowan, M.R., McGuigan, K., Davison, T.M., Hussain, A.M., Kafi, M., Matschoss, A. 1997. Relationships among calving season, heat load, energy balance and postpartum ovulation of dairy cows in a subtropical environment. *Anim. Reprod. Sci.* 47, 315-326.
- Kastelic, J.P., Ginther, O.J. 1989. Fate of conceptus and corpus luteum after induced embryonic loss in heifers. *JAVMA* 194, 922.
- Kindahl, H., Odensvik, K., Aiumlamai, S., Fredriksson, G. 1992. Utero-ovarian relationships during the bovine postpartum period. *Anim. Reprod. Sci.* 28, 363-369.
- Kinder, J.E., Bergfeld, E.G.M., Wehrman, M.E., Peters, K.E., Kojima, F.N. 1995. Endocrine basis for puberty in heifers and ewes. *J. Reprod. Fert. suppl.* 49, 393-407.
- Kruip, Th.A.M., Van der Werf, H.J., Wensing, Th. 1996. Energy balance in early lactation of high producing dairy cows and its relation to reproduction, health and welfare. In: Groen, A.F. and Van Bruchem, J. (Eds.), *Utilization of local feed resources by dairy cattle: Perspectives of*

- environmentally balanced production systems. Wageningen Pers, Wageningen, pp. 45-57.
- Kuenzel, W.J., Fraley, G.S. 1995. Neuropeptide Y: Its role in the neural regulation of reproductive function and food intake in avian and mammalian species. *Poult. Av. Biol. Rev.* 6, 185-209.
- Lamming, G.E., Mann, G.E. 1995. A dual role for progesterone in the control of cyclicity in ruminants. *J. Reprod. Fert. suppl.* 49, 561-566.
- Lucy, M.C., Staples, C.R., Thatcher, W.W., Erickson, P.S., Cleale, R.M., Firkins, J.L., Clark, J.H., Murphy, M.R., Brodie, B.O. 1992. Influence of diet composition, dry-matter intake, milk production and energy balance on time of post-partum ovulation and fertility in dairy cows. *Anim. Prod.* 54, 323-331.
- Lussier, J.G., Matton, P., Dufour, J.J. 1987. Growth rates of follicles in the ovary of the cow. *J. Reprod. Fert.* 81, 301-307.
- Madej, A., Kindahl, H., Woyno, W., Edqvist, L.-E., Stupnicki, R. 1984. Blood levels of 15-keto-13,14-dihydro-prostaglandin F2a during the postpartum period in primiparous cows. *Theriogenology* 21, 279-287.
- Northey, D.L., French, L.R. 1980. Effect of embryo removal and intrauterine infusion of embryonic homogenates on the lifespan of the bovine corpus luteum. *J. Anim. Sci.* 50, 298-302.
- Savio, J.D., Boland, M.P., Roche, J.F. 1990. Development of dominant follicles and length of ovarian cycles in post-partum dairy cows. *J. Reprod. Fert.* 88, 581-591.
- Senatore, E.M., Butler, W.R., Oltenacu, P.A. 1996. Relationships between energy balance and post-partum ovarian activity and fertility in first lactation dairy cows. *Anim. Sci.* 62, 17-23.
- Spencer, T.E., Ott, T.L., Bazer, F.W. 1996. T-Interferon: Pregnancy recognition signal in ruminants. *Proc. Soc. Exp. Biol. Med.* 213, 215-229.
- Spicer, L.J., Tucker, W.B., Adams, G.D. 1990. Insulin-like growth factor-I in dairy cows: relationship among energy balance, body condition, ovarian activity and estrous behaviour. *J. Dairy Sci.* 73, 929-937.
- Sreenan, J.M., Diskin, M.G. 1986. The extent and timing of embryonic mortality in the cow. In: Sreenan, J.M. and Diskin, M.G. (Eds.), *Embryonic Mortality in Farm Animals*. Martinus Nijhoff Publishers, Dordrecht, pp. 1-11.
- Staples, C.R., Thatcher, W.W., Clark, J.H. 1990. Relationship between ovarian activity and energy status during the early post partum period of high producing dairy cows. *J. Dairy Sci.* 73, 938-947.
- Thatcher, W.W., Wilcox, C.J. 1973. Postpartum estrus as an indicator of reproductive status in the dairy cow. *J. Dairy Sci.* 56, 608-610.
- Thatcher, W.W., Meyer, M.D., Danet-Desnoyers, G. 1995. Maternal recognition of pregnancy. *J. Reprod. Fert. suppl.* 49, 15-28.
- Villa-Godoy, A., Hughes, T.L., Emery, R.S., Chapin, L.T., Fogwell, R.L. 1988. Association between energy balance and luteal function in lactating dairy cows. *J. Dairy Sci.* 71, 1063-1072.
- Whitaker, D.A., Smith, E.J., daRosa, G.L., Kelly, J.M. 1993. Some effects of nutrition and management on the fertility of dairy cattle. *Vet. Rec.* 133, 61-64.
- Yu, W.H., Kimura, M., Walczewska, A., Karanth, S., McCann, S.M. 1997. Role of leptin in hypothalamic-pituitary function. *Proc. Natl. Acad. Sci. USA* 94, 1023-1028.
- Zavy, M.T. 1994. Embryonic mortality in cattle. In: Zavy, M.T. and Geisert, R.D. (Eds.), *Embryonic Mortality in Domestic Species*. CRC Press, Boca Raton, pp. 99-140.