BIOTROPIA VOL. 13 NO. 2,2006 : 75 - 84

PREOVULATORY CHANGES AND OVULATION IN CATTLE UNDERGOING SPONTANEOUS OR CLOPROSTENOL-INDUCED LUTEOLYSIS

BAMBANG PURWANTARA[1,3], RENE H0IER[2], METTE SCHMiDT[2], AND TORBEN GREVE[2]

[1] Department of Veterinary Clinic, Reproduction and Pathology, Faculty of Veterinary Medicine, Bogor Agricultural University, Darmaga 16680 Bogor, Indonesia
[2]Department of Clinical Studies, Section Reproduction Royal Veterinary and Agricultural University, Bulowsvej 13, 1870 Frederiksberg C. Denmark
[3]SEAMEO BIOTROP, Jl. Raya Tajur Km 6, P.O. Box 116Bogor, Indonesia

ABSTRACT

The follicular population, diameter of the ovulatory and subordinate follicles, corpus luteum (CL) size, concentration of progesterone and estradiol-17p were studied following spontaneous or cloprostenol-induced luteolysis. A total of 14 heifers received cloprostenol treatment on Day 9-11 of the cycle to synchronize their estrus. Subsequently, they were divided into two groups, one group which was allowed to undergo spontaneous luteolysis and the other group in which estrus was induced from days 9 to 12. In the induced-group, transrectal ultrasonography were performed daily started two days prior to injection until the onset of estrus. In the spontaneous-group, ultrasonography was done daily from day 15 until the onset of estrus. In both groups scanning were performed every 4 h from the onset of estrus until ovulation was ascertained. Small (SF, 2-4 mm), medium (MF.5-9 mm) and large (LF,>9mm) size follicles were recorded. The diameter of largest and subordinate follicles were measured and blood were drawn from jugular vein at approximately around scanning and the plasma were used for measurement of progesterone (P4) and estradiol-17p (E2) concentration. There was no different in term of number of SF, MF and LF (P>0.05) between the two groups. Similarly, no effect of side (left vs. right ovary) and CL position (ipsi- vs contralateral to the ovary) was found (P>0.05). However, it was demonstrated that mean number of ovulatory follicles was higher (PO.01) in the spontaneously ovulating group while the regressing-CL size was larger in the cloprostenol induced animals (P<0.05). Occurrence of time of ovulation in relation to initial signs of estrus was observed in both groups of animals. This variation could be attributed to the existence of a large preovulatory follicle which enhanced the time ovulation. Conversely, when subordinate follicles showed grow up and replace the large follicle the interval from heat to ovulation was prolonged. Progesterone experienced a more steep decrease than the spontaneous group of animals and a positive correlation was observed between the diameter of CL and the P4 concentration. For E2 there was a positive correlation between the E2 and follicular size. It is concluded that the variation in follicular among animals contributed to the variability in timing of ovulation, particularly prostaglandin-induced animals. The diameter of ovulatory follicle in spontaneous group was larger as compared to induced-group.

Key words : follicular development, ovulation, cloprostenol,cattle reproduction

INTRODUCTION

Preovulatory follicular development and its regulation in cattle has so far remained unclear (Assey *et al.* 1993; Lindsay *et al.* 1996; Ireland *et al.* 2000; Evan 2001; Burn *et at.* 2005). Detailed studies of the pattern of follicular development during the estrous cycle has been based on slaughter house material (Rajakoski 1960; Dufour *et al.* 1972) and ultrasonography (Pierson and Ginther 1989a; Pierson

Corresponding author: b.purwantara@biotrop.org

and Ginther 1989b; Fortune *et al.* 1988; Rivera *et al.* 2001; Ginther *et al.* 2001; Webb *et al.* 2003) However, the processes by which the ovulatory follicles is selected is still not clear (Staigmiller and England 1982; Pierson and Ginther 1988 and Quirk *et al.* 1986; Mihm *et al.* 2000; Evan 2003; Kobayashi *et al.* 2006), although india ink marking of preovulatory follicles combined with cauterization of certain larger follicles (Matton *et al.* 1981) has improved the understanding of the underlying mechanisms.

The development of clinical ultrasonogaphy (Quirk *et al.* 1986; Pierson and Ginther 1989a; Pierson and Ginther 1989b; Savio *et al.* 1988; Sirois and Fortune 1988; Purwantara *et al.* 1993; Purwantara *et al.* 1994; Ginther et al. 2001; Frike *et al.* 2002) which allows visualization of ovarian follicles over time, has given even a better insight. It is, however, still an open question when the follicle destined to ovulate can be recognized and how this affect the subordinate follicular population. The timing of ovulation in cattle has in most studies been determined in spontaneous non superovulated cows/heifers either by rectal palpation (Schams *et al.* 1977) or by ultrasonography (Larsson 1987), and in superovulated cattle (Savio *et al.* 1990; Fortune *et al.* 1991; Purwantara *et al.* 1994). However, no detailed studies are available concerning a possible relationship between the occurrence of ovulation and preovulatory hormonal parameters (progesterone and estradiol 17p in neither spontaneous nor prostaglandin induced luteolysis and ovulation.

The aim of this study was, therefore during the preovulatory periods in spontaneous vs. induced luteolysis (1) to monitor the development of ovulatory and non-ovulatory follicles at various times prior to ovulation (2) to characterize the regression of the corpus luteum, and (3) to determine progesterone (P4) and estradiol (E2) profiles and relate those to the time of ovulation.

MATERIALS AND METHODS

Animals

Fourteen heifers of mixed beef cattle breed between 2-3 years of age and weighing between 300-500 kg were used in the experiment. The animals had normal estrous cycles at least once prior to the study. Subsequently, the animals were divided equally into two groups, the spontaneous-group and the induced-group. Spontaneous-group: Following induced estrus using a single injection of cloprostenol (Estrumat Vet., Cooper Animal Health Ltd., Denmark) and ovulation, the animals were left alone for approximately 19-20 days at which time close observation for a spontaneous heat was performed three times daily. Induced-group: On day 9-12 of estrous cycle (day estrus = day 0) the heifers received a single injection of cloprostenol (Estrumat Vet., Cooper Animal Health Ltd., Denmark) to induce estrus and ovulation.

Ultrasonography

A real-time B-mode diagnostic ultrasound equipments (Concept Ultrasound Scanner, Dynamic Imaging Ltd., UK) equipped with a linear array 7.5 MHz transrectal transducer was used. Detailed procedures of the ultrasonographic examination of the ovaries were performed as described earlier by Purwantara *et al.* (1994) and the schedule was as follows: In the spontaneous-group, scanning was performed daily from Day 15 of the estrous cycle until onset of heat and then every 4 h. In the induced-group daily ultrasonographic scanning were done two days prior to cloprostenol injection and until the onset of heat. From the onset of heat and until ovulation was confirmed the animals were scanned every 4 h.

The number of small (SF, 2-4 mm), medium (MF, 5-9 mm), and large (LF, >10 mm) size ovarian follicles were counted and the diameter of the largest, subordinates and the diameter of corpus luteum were measured by using built-in integral caliper. Corpus luteum integrity were scored as 3 (highly intact), 2 (moderate) and 1 (less intact, difficult to discriminate) depending on the gray shade and the demarcation line between the corpus luteum and the stroma. Each ovarian scan was recorded by means of video recorder and diagram of each ovary were drawn. Individual preovulatory and its subordinate follicles were closely examined. Ovulation was defined as the disappearance of the largest non-echogenic area (dominant follicles) between two consecutive examinations.

Blood sampling and hormone measurement

Daily blood sampling were drawn from jugular or coccygeal vein using heparinized vacuum tube. Blood plasma was separated by centrifugation at 3000 rpm for 15 min and stored at -20°C until analysis was performed. Progesterone (P4), estradiol-17p (E2) and LH concentration were analyzed in all samples by RIA according to H0ier (1989), respectively. The inter- and intra-assay coefficient of variance (CV) of P4 measurement was 3-10% and 7-15% respectively, and <10% for E2 depending on the position of displacement of curve. The sensitivity (least detectable concentration) was 0.25 ng/ml and 4 pg/ml for P4 and E2 measurement, respectively.

Statistical analysis

Data were analyzed using general linear model for repeated measurement analysis of variance of Statistical Analyses System (SAS 1986). The effect of group (spontaneous vs. induced) and day on mean number of SF, MF, LF and total follicle (TF) were determined. The similar effect were examined on the diameter of ovulatory follicle and its subordinate, corpus luteum diameter, progesterone (P4) and estradiol-17p (E2) concentration. Regression analysis and Pearson correlation coefficient were determined to relate CL diameter and P4. Correlation analysis were also performed to examine relationships between the occurrence of ovulation and the rate of CL regression and the changes on diameter of ovulatory follicle.

RESULTS AND DISCUSSION

Occurrence of estrus was subject to a great deal of individual variation but it was in general observed on between day 18 and 23 of the estrous cycle in heifers undergoing spontaneous luteolysis and on the third day after cloprostenol injection. In the spontaneous-group, the inter-ovulatory interval ranged from 19 d 2 h to 23 d 10 h. In the induced-group, ovulations occurred from PG-injection, ranging from 64 (62-66) h to 126 (124-128) h. In 4 animals where the LH surge was clearly identified, the interval between LH surge to ovulation ranged from 24 to 26 h.

A pronounced individual variation occurred in terms of the time which lapsed between prostaglandin injection (induced-group) or CL regression (spontaneous-group) and subsequent ovulation. It is anticipated at least in the induced-group that the stage of follicular development plays an important role on these differences. Savio *et al.* (1990) and Ginther *et al.* (2001) observed that the majority of ovulation originated from the follicles which was dominant on day 7 at the time when prostaglandin was injected. Moreover, Fortune *el al.* (1991) and Evan (2003) confirmed that the majority of the dominant follicle undergo ovulation when luteolysis was induced during the growing or early plateau phase. In contrast, when prostaglandin was injected during the late plateau phase or atresia phase, the largest follicles failed to ovulate and its subordinate became the ovulatory follicles.

In two heifers ovulating within 62-66 h (<3 days) after cloprostenol injection, the ovulatory follicle was generally not difficult to determine as a growing larger follicle where the subordinate had diameters less than 5 mm during the last 3 days prior to ovulation. No other large and medium size follicle were detected in these animals, the follicle which became the largest follicle during the 3 days prior to ovulation. This follicle underwent ovulation while the other became atretic. The other two of these late-ovulating groups developed two large follicles >10 mm which both increased in size until ovulation. Ovulation occurred from the follicles which remained the largest during the last 5 days prior to ovulation. In the animals where ovulation occurred within 3 and 4 days after cloprostenol injection shift in dominance was observed only in one animal, while in the remaining, the dominant ovulatory follicles suppressed their subordinates which underwent atresia.

Evidence from this study showed that a shorter interval between injection of cloprostenol and ovulation occurred when a single large follicle with no medium size subordinate follicles were present during the period of luteolysis. In contrast, the interval was longer when the dominant follicle had to be or was replaced by one of the subordinate or when more than two large follicles developed during the phase of luteolysis. This is in agreement to Larsson (1987) who found an increase in the length of proestrus period when 2 follicles greater than 10 mm in diameter were present in that period. Some developmental changes during the transitional phase from early to late plateau phase of growth, characterized by a loss in the ability of the dominant follicle to ovulate may contribute to this variation (Sirois and Fortune 1990; Ginther *et al.* 2001; Evan 2003; Burn *et al.* 2005).

Ovulation was not dependant upon the side. Ovulations occurred on the right ovary in 55 % of the induced-group (n=ll) and *in 56 %* of the spontaneous-group

(n=14). The trend was not statistically significant (P>0.05). Ovulations occurred ipsi-lateral or contra-lateral to the corpus luteum in 55% or 45%, respectively of the spontaneous ovulations and 64% or 36%, respectively of the induced ovulations. One heifer developed two large size growing follicles on the same ovary and they both ovulated. These ovulations occurred within a 4 h interval and no extended preovulatory period was observed. No significant difference were demonstrated on the mean number of SF, MF and LF between the groups (P>0.05). It was noticeable as seen on Fig. 1 (A), that the ovulatory follicle in the spontaneous-group attained a larger mean diameter than the ovulatory follicles in the induced-group. Mean diameter of ovulatory follicle was different between group (P<0.01) and day (P<0.01). In the spontaneous-group as seen on Fig. 1 (B), the mean diameter of subordinate follicles remained fairly stable until 2 days prior to ovulation at which time they decreased, a pattern which was contrary to the induced group

The interovulatory interval was subject to a wider variation in the group undergoing spontaneous ovulation. Perhaps, the number of waves has influenced this variation. It was reported by Ginther *et al.* (1989a) that the length of the interovulatory interval was in average of 2.4 days longer in 3-wave than in 2-wave cycles. Other studies on the other hand (Savio *et al.* 1988; Sirois and Fortune 1988) were unable to find differences in the interovulatory interval between the 2-wave and 3-wave pattern animals. Since this study was limited to the preovulatory period, these contradictory findings could not be confirmed. In this particular period, however, it was reported that the length of interval from luteal regression to ovulation (Ginther *et al.* 1989a; Ginther *et al.* 1989b) and growth of follicle during luteolysis (Savio *et al.* 1988; Sirois and Fortune 1988) did not differ between 2-wave and 3-wave pattern animals.

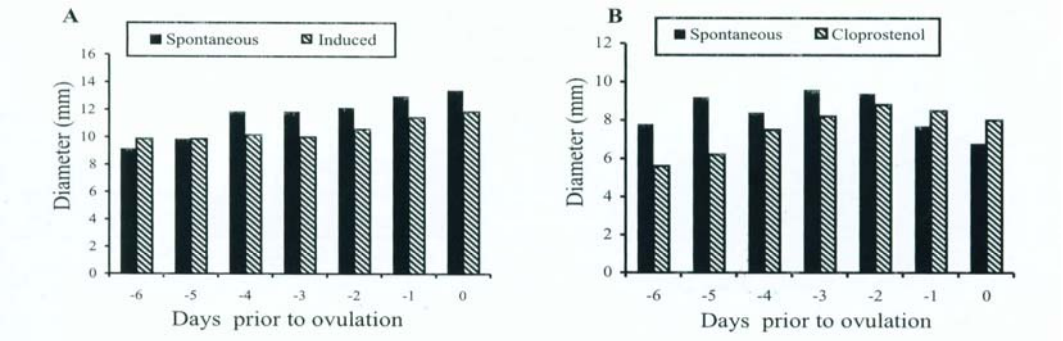

Figure 1. Mean diameter of ovulatory (A) and subordinate (B) follicle during the preovulatory period.

The number of small, medium and large size follicle was not different between the two groups and days. This indicated a similar pattern of folliculogenesis in spontaneous and induced ovulations. In addition, during luteolysis no major changes

in follicular population was observed except the disappearance of ovulatory follicles at the time of ovulation.

No ovulation occurred when the diameter of follicle was less than 10 mm. The larger mean diameter of ovulatory follicles which was observed in spontaneous-group is not in agreement to Quirk *et al.* (1986) who found no significant difference of ovulatory follicle development in spontaneous vs. cloprostenol-induced ovulation. This emphasizes that the size alone is not a good indicator of readiness to undergo ovulation. An interesting pattern was observed concerning the mean diameter of subordinate follicles in the spontaneous and induced-group. In the spontaneous group, the mean diameter remained stable until proestrus and then decreased dramatically, while in the induced -group the subordinate follicles tended to increase in size until estrus and then decreased slightly. It is speculated, therefore, that the ovulatory follicles in the spontaneous-group had exhibited a clear dominancy several days before estrus, while those in the induced-group was not selected until the latest stage of the cycle.

In this study ovulations occurred with an equal frequency on left and right ovary. This is different from previous studies (Rajakoski 1960; Pierson and Ginther 1989b; Purwantara *et al.* 1992) which reported that ovulations occurred more frequently on the right than the left ovary. In addition, we found no ipsi- or contralateral effect of CL on ovulation in the spontaneous-group and only a slightly greater number of ovulation from ipsilateral CLs in the induced-group. This is conflicting to Rajakoski (1960) who found a significant greater number of ovulation occurred from CL bearing ovary.

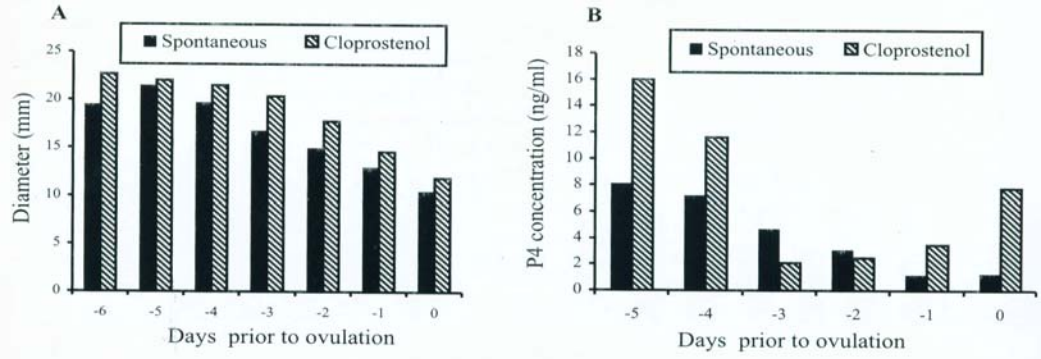

Figure 2. Mean diameter of corpus luteum (A) and concentration of P4 (B) during preovulatory period

As depicted in Fig. 2 (A), the mean diameter of corpus luteum was affected significantly by the group ($P < 0.01$) and the induced-group maintained a higher diameter. A similar trend was indicated on the effect of day ($P<0.01$).

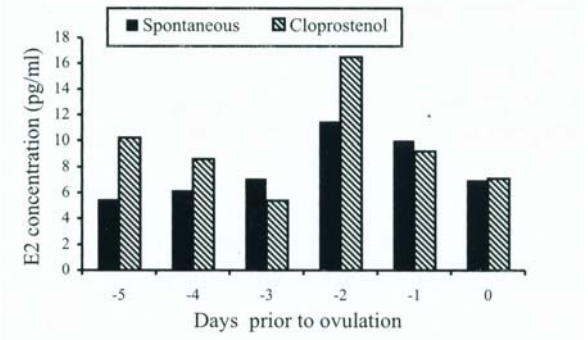

Figure 3. Mean concentration of E2 during preovulatory period.

The CL was scored as 3 at the day of cioprostenol injection, then it changed to 2 sometime between the second and third days and reached score 3 at the day - or 1 day before -ovulation. In the spontaneous group score 3 existed 5-4 days before ovulation, then attained score 2 during the following 2-3 days and reached score 1 at 1-2 days prior to and until the day of ovulation. The mean concentration of P4 decreased following cioprostenol injection as seen on Fig. 2 (B) and more dramatically in the induced-group. However, in some heifers, P4 concentration did not decline to reach a level under 1 ng, which is defined as the level compatible with luteal regression. On the other hand, the concentration of E2 increased and reached a maximum level 48 h prior to ovulation, and then decreased (Fig. 3).

A positive correlation was found to exist between P4 concentration and CL diameter during the regression period (r=0.51, PO.01). Similarly was plasma P4 and CL integrity (r=0.49, P<0.01), and CL diameter and CL integrity (r=0.86, PO.01). The mean diameter of the regressing CL was larger in the induced-group compared to spontaneous-group and although subject to great variation, the concentration of P4 was also greater in induced-group until 3 days prior to ovulation. It is interesting to note that the dramatic decrease of P4 in the induced-group between day *5* and day 3 prior to ovulation was not accompanied with a similar trend in the decrease of CL diameter, but rather related to the CL integrity. We speculate that the decrease of P4 concentration during luteolysis in the induced-group occurred ahead of decrease in CL diameter, and was different from the spontaneous luteolysis. Concentration of E2 reached the peak 2 days prior to ovulation or about 1 day before the LH surge which it is in agreement to other studies (Harrison *et al.* 1985; Dutchen *et al.* 1994; Rhodes *et al.* 1995; Singh *et al.* 1997; Valdez *et al.* 2005).

CONCLUSIONS

It was concluded that the timing of ovulation following luteolysis varied between animals both in spontaneous and induced- group. This variation was predominant in the induced-group and it was shorter when the single large

preovulatory follicle existed, and tended to occur later when a subordinate follicle had to replace a large follicle and became the preovulatory follicle. No effect of side (left or right ovary) and ipsi- or contralateral position of CL to the ovulatory follicle was found. Population of small, medium and large size follicles was not different between induced and spontaneous-group. However, mean diameter of ovulatory follicle was larger in spontaneous compared to induced-group. Moreover, the diameter of subordinate follicles and CL regression varied between the two treatment groups.

ACKNOWLEDGMENTS

This study was supported by Animal Biotechnology Research Center, Royal Veterinary and Agricultural University Copenhagen Denmark and Danida. The authors also would like to thank Mr. Ben S01oy for providing assistance for the hormonal analysis.

REFERENCES

Assey, R.J., B. Purwantara, T. Greve, P. Hyttel and M. Schmidt. 1993. Corpus luteum size and plasma progesterone levels in cattle after induction of luteolysis. Theriogenology, 39 (6): 1321-1330. Burns, D.S., F. Jimenez-Krassel, J.L.H. Ireland, P.O. Knight and J.J. Ireland. 2005. Numbers of antral follicles during follicular waves in cattle: Evidence for high variation among animals, very high repeatability in individuals, and an inverse association with serum follicle-stimulating hormone Concentrations. Biol Reprod ., 73(1): 54 - 62. Duchens, M., M. Forsberg, L.E. Edqvist, H. Gustafsson and H. Rodriguez-Martinez. 1994. Effect of induced suprabasal progesterone levels around estrus on plasma concentrations of progesterone, estradiol-178, and LH in heifers. Theriogenology, 42:1159-1169 Dufour, J., H.L. Whitmore, O.J. Ginther. and L.E. Casida. 1972. Identification of the ovulating follicles by its size on different days of estrous cycle in heifers. J. Anim. Sci., 24: 85-87. Evan, A.C.O. 2003. Characteristic of ovarian follicle development in domestic animals. Reprod. Domest. Anim., 38 (4): 240-246 Fricke, P.M. 2002. Scanning the Future—Ultrasonography as a reproductive management tool for dairy cattle. J.Dairy Sci., 85:1918-1926 Fortune, J.E., J. Sirois and S.M. Quirk, 1988. The growth and differentiation of ovarian follicles during the bovine estrous cycles. Theriogenology, 22: 95-109. Fortune, J.E., J. Sirois, A.M. Turzillo and M. Lavoir. 1991. Follicle selection in domestic ruminants. J. Reprod. Fertil. Suppl., 42: 187-198. Ginther, O.J., L. Knopf and J.P. Kastelic, 1989. Temporal associations among ovarian events in cattle during oestrous cycles with two or three follicular waves. J. Reprod. Pert., 87: 223-230. Ginther, O.J., J.P. Kastelic and L. Knopf, 1989. Composition and characteristics of follicular waves during the bovine estrous cycle. Anim. Reprod. Sci., 20: 187-200. Ginther, O.J., E.L. Gastal, M.O. Gastal, D.R. Bergfelt, F.X. Donadeu and K. Kot. 2001. Follicular selection in monovular species. Biol. Reprod., 65 (3): 638-647. Harrison, L.M., R.D. Randel, D.W. Forrest, J.G. Belts, W.D. Humprey and D.R. Hardin. 1985. Cloprostenol induced luteal regression in the beef cow. I. Ovarian response and endocrine changes. Theriogenology, 23: 511-521.

H0ier, R. 1989. Improvement of a direct, sensitive progesterone ELISA for analysis of samples from domestic animals: Ion exchange purification of progesterone: -horseradish peroxidase conjugates (Technical Report, Department of Clinical Studies, Section of Reproduction - RVAU Denmark). Ireland, J.J. 1987. Control of foilicular growth and development. J. Reprod. Fertil., 35 (Suppl.): 39-54. Ireland, J.J., M. Mihm, E. Austin, M.G. Diskin and J.F. Roche. 2000. Historical perspective of turn over of dominant follicles during the bovine estrous cycle : Key concepts, studies, advancements and terms. J Dairy. Sci., 83 (7): 1648-1658. Larsson, B. 1987. Determination of ovulation by ultrasound examination and its relation to the LH-peak in heifers. J. Vet. Med. A., 34: 749-754. Lindsey B.R., M.E. Wehrman, E.J. Melvin, J.A. Quintal, F.N. Kojima, E.L. Zanella, K.E. Fike, E.G.M. Bergfeld and J.E. Kinder. 1996. Ovarian foilicular growth and FSH secretion following a timed injection of progesterone in cows. Theriogenology, 45:325 (abstract) Matton, P., V. Adelakoun, Y. Couture and J.J. Dufour, 1981. Growth and replacement of the bovine ovarian follicles during the estrous cycle. J. Anim. Sci. 52: 813-820. Mihm, M., E.J. Austin. T.E.M. Good, J.L.H. Ireland, P.O. Knight, J.F. Roche and J.J. Ireland. 2000. Identification of potential intrafollicular factors involved in selection of dominant follicles in heifers. Biol. Reprod., 63 (3): 811-819. Pierson, R.A. and O.J. Ginther. 1987a. Foilicular populations during the estrous cycle in heifers: I. The influence of day. Anim. Reprod. Sci., 14: 165-176. Pierson, R.A. and O.J. Ginther. 1987b. Foilicular populations during the estrous cycle in heifers: II. Influence of right and left sides and intraovarian effect of corpus luteum. Anim. Reprod. Sci., 14: 177-186. Pierson, R.A. and O.J. Ginther. 1988. Foilicular populations during the estrous cycle in heifers: III. Time of selection of the ovulatory follicle. Anim. Reprod. Sci., 15: 81-95 Purwantara, B., RJ.Assey, M. Schmidt. P. Hyttel and T. Greve. 1992. Dynamics of the first foilicular wave in cattle following cloprostenol-mduced luteolysis. Proceedings ICARThe Hague 1992. Purwantara, B. M. Schmidt, T. Greve and H. Callesen. 1993. Foilicular dynamics prior to and during superovulation in heifers. Theriogenology, 40:913-921. Purwantara, B., H. Callesen and T. Greve. 1994. Characteristics of ovulations in super-ovulated cattle. Anim. Reprod. Sci., 37: 1-5.
Quirk, S.M., G.J. Hickey, and J.E. Fortune. 1986. Growth and regression of ovarian follicles during the foilicular phase of the oestrous cycles in heifers undergoing spontaneous and PGF 2a-induce luteolysis. J. Reprod. Fertil., 77: 211 (abstract).
Rajakoski, E. 1960. The ovarian foilicular system in sexually mature heifers with special reference to seasonal, cyclical and left-right variations. Acta Endocrinol. 34 Suppl. 52. Rhodes P.M., L.A. Fitzpatrick, K.W. Entwistle and J.E. Kinder. 1995. Pulsatile hormone secretion during the first ovarian wave in *Bos indicus* heifers. J. Reprod. Fertil., 49:523-526. Rivera, G.M. and J.E. Fortune. 2001. Development of codominant follicles in cattle is associated with a follicle stimulating hormone-dependent insulin-like growth factor binding protein-4 protease. Biol. Reprod., 65 : 112-118.
SAS, 1986. SAS/STAT User's Guide Version 6.01. SAS Institute Inc. Gary NC. Savio. J.D., L. Keenan, M.P. Boland and J.F. Roche. 1988. Pattern of growth of dominant follicles during the estrous cycles of heifers. J. Reprod. Ferti., 83:663-671. Schams, D., E. Schallenberger, B. Hoffmann and H. Karg. 1977. The oestrous cycle of the cow: Hormonal parameters and time relationships concerning oestrus, ovulation, and electrical resistance of the vaginal mucus. Acta Endocrinol., 86: 180-192. Singh, J., R.A. Pierson, and G.P. Adams. 1997. Ultrasound image attributes of the bovine corpus luteum: structural and functional correlates. J. Reprod. Fertil., 109:35^4. Sirois, J. and J.E Fortune. 1988. Ovarian foilicular dynamics during the estrous cycle in heifers monitored by real-time ultrasonography. Biol. Reprod., 22. 308-317. Staigmiller, R.B. and B.C. England. 1982. Folliculogcnesis in the bovine. Theriogenology, 17: 43-52.

Valdez K.E., S.P. Cuneo, P.J. Garden and A.M. Turzillo. 2005. The role of thecal androgen production in the regulation of estradiol biosynthesis by dominant bovine follicles during the first folicular wave. J Anim. Sci., 83: 597-603 Webb, R., B. Nicholas, J.G. Gong, B.K. Campbell, C.G. Gutierrez, H.A. Garverick and D.G. Armstrong.
2003. Mechanism regulating follicular development and selection of dominant follicle. Reprod. Suppl., 61:71-90.