Superovulation following follicular synchronization with GnRH at random stages of the oestrous cycle in heifers

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ABSTRACT: The objective of this study was to develop a superovulatory program based on synchronization of follicular waves with GnRH which could be applied regardless of the stage of the oestrous cycle. 36 heifers were subjected to this experiment and GnRH (Cystorelin, 200 μg) was applied between Days 0 and 7 (n = 15), 8 and 12 (n = 8) or 13 and 20 (n = 13) of the oestrous cycle. Four days after GnRH treatment, all follicles ≥ 6 mm of heifers (n) were either punctured (n = 21) or left intact (n = 15). All heifers were superstimulated from Day 6 to Day 10 after GnRH treatment with 320 mg Folltropin-V. In parallel, 21 heifers were superstimulated in a conventional manner (Days 8 to 12) and were used as controls. The homogeneity of follicular inventories among Stage-groups occurred within 4 days of GnRH treatment for follicles ≥ 7 mm but only 2 days after follicular puncture for follicles 4 to 6 mm. In response to the follicular puncture, the mean number of follicles 4 to 6 mm increased in heifers of the punctured group (P < 0.01). Following the superstimulation, the follicular (P < 0.01) and ovulatory (P < 0.01) responses were higher in the punctured group than in the nonpunctured group. The *in vivo* production of transferable embryos in the punctured group was similar to that of the nonpunctured group but it was lower (P < 0.01) than in heifers of the control group. In conclusion, results from the present study indicate that regardless of the stage of the oestrous cycle, the homogeneity of follicular inventories following the follicular synchronization is obtained using GnRH treatment and follicular puncture. The *in vivo* production of embryos was severely compromised in the present study with heifers. Causes of such reduction in the *in vivo* production of embryos are still unknown.

Keywords: oestrous cycle; GnRH; superstimulation; follicular puncture; heifer

For bovine embryo production, superstimulation is usually initiated between Days 8 and 12 of the oestrous cycle. It has been reported that approximately 25 to 50% of cows display low embryo responses (Greve et al., 1995) when superstimulation is initiated between these days of the oestrous cycle, most likely due to inadequate or less than optimal follicular condition in a significant proportion of the cows. Indeed, on the day of the initiation of superstimulation, the presence of a dominant follicle decreases the superstimulatory response (Guilbault et al., 1991; Wehrman et al., 1996) while that of a high number of recruitable follicles is associated with significant increases of these responses (Romero et al., 1991; Bungartz and Niemann, 1994).

A large body of evidences indicates that variability of superovulatory responses in cattle is due to heterogeneity of the follicular status among animals at the time of the initiation of superstimulation (Guilbault et al., 1991; Romero et al., 1991; Huhtinen et al., 1992; Bungartz and Niemann, 1994; Wehrman et al., 1996; Kohram et al., 1998a; Baracaldo et al., 2000; Andrade et al., 2002). Homogeneity of fol-

licular inventories, at least in terms of follicles ≥ 7 mm, occurs following the treatment with GnRH (Twagiramungu et al., 1995; Kohram et al., 1998a). Based on this concept, a new approach has been developed (Kohram et al., 1998b) whereby GnRH is used at two different stages of the oestrous cycle to elicit the emergence of a follicular wave in a synchronous fashion in a group of cows and to expose a dominant follicle for puncture at a predictable time (i.e. 4 days after GnRH). The effect of follicular synchronization with GnRH on hormonal and follicular response prior to and during superovulation was described (Kohram et al., 1998b) but its effect on embryo production has not been clearly defined yet.

The objective of this study was to determine the effect of follicular synchronization with GnRH and of follicular puncture at any stage of the oestrous cycle prior to superovulation on the follicular status at the initiation of superstimulation and on *in vivo* production of bovine embryo. In this study the *in vivo* production of bovine embryos was severely reduced.

MATERIAL AND METHODS

Animals and treatment

The follicular status was altered by an *i.m.* injection of GnRH (Cystorelin, 200 μg, Sanofi, Quebec, Canada) in 36 cycling Holstein heifers (14 to 24 months of age). GnRH was applied between Days 0 and 7 (Stage-group 1, n = 15), Days 8 and 12 (Stage-group 2, n = 8) or Days 13 and 20 (Stage-group 3, n = 13) of the oestrous cycle (oestrus = Day 0). Four days after GnRH treatment, all heifers were randomly assigned to two groups in which follicles ≥ 6 mm were either punctured (GnRH-punctured group; n = 21) by follicular aspiration according to the method previously described (Pieterse et al., 1991) or left intact (GnRH-non-punctured group; n = 15). All heifers were superstimulated from Day 6 to Day 10 after GnRH treatment with 320 mg Folltropin-V (Vetrepharm Canada Inc., London, Ontario, Canada) given twice daily at decreasing doses over four days. Two injections of Cloprostenol (Coopers Agropharm, Ajax, Ontario, Canada; 500 μg/injection) were given intramuscularly with the fifth and the sixth injections of Folltropin-V. All heifers were artificially inseminated at oestrus and 12 h later with a single straw of semen from bulls of proven fertility. Seven to eight days after insemination, the number of CL was evaluated by ultrasonography and palpation. Ova and embryos were collected by a nonsurgical procedure evaluated and classified as Grade 1 (excellent or very good), Grade 2 (good), Grade 3 (fair), degenerated and unfertilized ova (IETS, 1990). Numbers of Grade 1 and 2, and of transferable embryos (Grade 1, 2 and 3) were also determined.

In parallel, 21 heifers raised under the same management conditions were superstimulated in a conventional manner with 320 mg Folltropin-V given between Days 8 and 12 of the oestrous cycle (oestrus = Day 0). This group of heifers was used as a control for the number of CL (evaluated by palpation) and number of embryos.

The ovaries of all GnRH-treated heifers were examined by ultrasonography with a real-time linear scanning ultrasound diagnostic system (LS-300-A, Tokio Keiki Co., Tokyo, Japan; 7.5 MHz transducer) on Days 0 (GnRH treatment), 4 (follicular puncture), 6 (initiation of superstimulation), 10 (oestrus), and 17 or 18 (embryo collection) after GnRH treatment. All follicles larger than 3 mm were counted and classified according to their diameter in one of the following classes: 4 to 6 mm, and ≥ 7 mm (Kohram et al., 1998a).

Blood samples were collected in all GnRH-treated heifers from the jugular vein into heparinized tubes immediately before ultrasonography was performed. Plasma was harvested within 1 h of collection and stored at –20°C until assayed for progesterone or estradiol. Concentrations of progesterone and estradiol were determined by radioimmunoassay as previously described (Guilbault et al., 1988). Assay sensitivity for estradiol was 1.9 pg per ml with the extraction efficiency of 87%. Intra- and inter-assay coefficients of variation were 8% and 10%, respectively. Assay sensitivity for progesterone was 15.6 pg/ml with the extraction efficiency of 92%. Intra- and inter-assay coefficients of variation were 9.3% and 10%, respectively.

Statistical analysis

The mean number of follicles (i.e. 4 to 6 mm, and ≥ 7 mm), as well as estradiol and progesterone concentrations before the initiation of superstimulation were compared during 2 separate periods: (1) Day 0 to Day 4 (before follicular puncture), to analyze the

effect of GnRH treatment on the follicular and hormonal status before follicular puncture; and (2) Day 4 to Day 6 (before superstimulation), to analyze the effect of follicular puncture on the follicular dynamics and hormonal changes before the initiation of superstimulation. These data were analyzed by the least squares analysis of variance using the general linear model (GLM) procedure of SAS. The multivariate analysis included sources of variation due to the stage of oestrous cycle at the time of GnRH treatment, treatment (puncture vs. nonpuncture, between Day 4 and Day 6), days (repeated measures) and their interactions (MANOVA; SAS, 1985).

The mean superovulatory responses (i.e. number of follicles ≥ 7 mm at oestrus, number of CL at the time of embryo collection, number of transferable embryos, number of degenerated embryos, and number of unfertilized ova) as well as concentrations of estradiol at oestrus and of progesterone at the time of embryo collection were compared between the heifers. These data were analyzed by one-way analysis of variance using the general linear model (GLM) procedure of SAS. The model included sources of variation due to the stage of oestrous cycle at the time of GnRH treatment, treatment (puncture vs. nonpuncture) and their interac-

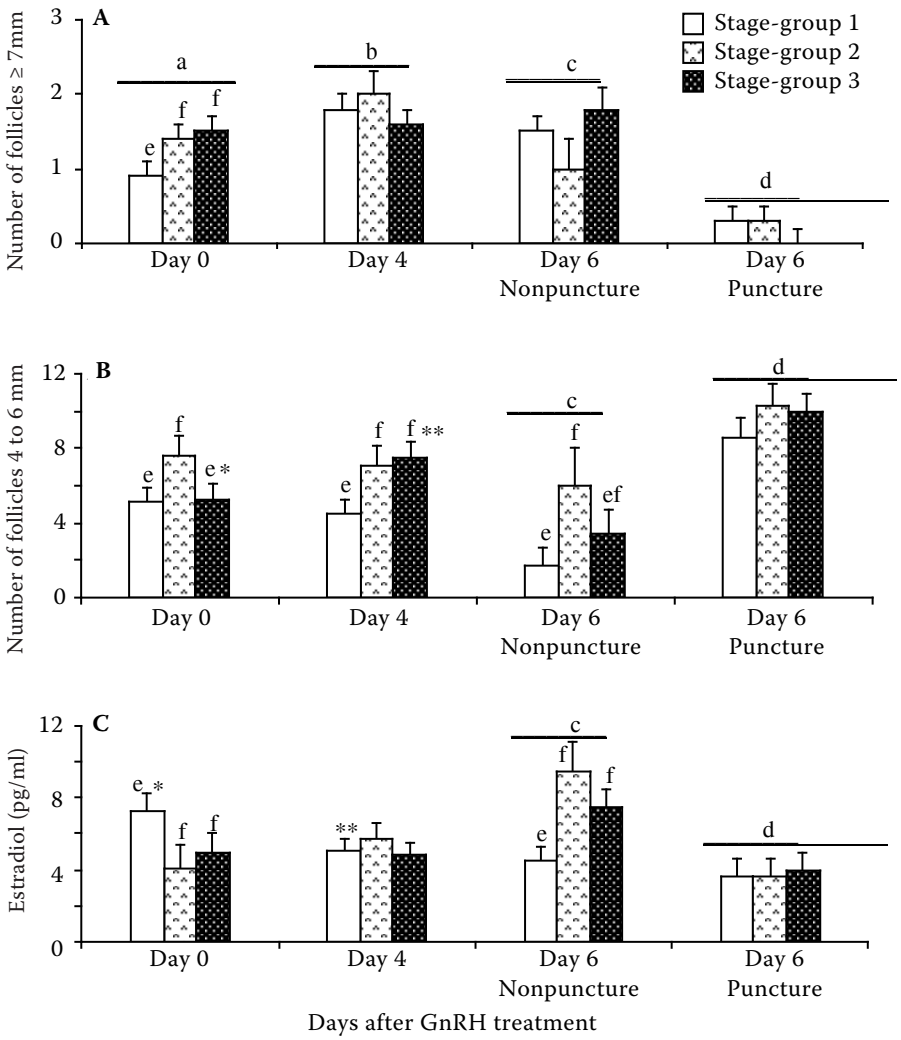

Figure 1. The mean number of follicles ≥ 7 mm (panel A) and 4 to 6 mm (panel B) and concentrations of estradiol (panel C) on Days 0 (GnRH treatment), 4 (follicular puncture or nonpuncture), and 6 (initiation of superstimulation) after GnRH treatment in three groups of heifers. GnRH was given between Days 0 and 7 (Stage-group 1; n = 15), 8 and 12 (Stage-group 2; n = 8) or 13 and 20 (Stage-group 3; n = 13) of the oestrous cycle (oestrus = Day 0); four days after GnRH treatment (Day 4), the follicles ≥ 6 mm were punctured (n = 21) or left intact (n = 15); different superscripts on the bars indicate a significant difference (P < 0.05) between days (a, b), puncture and nonpuncture Groups (c, d), Stage-groups (e, f), and interaction of Days × Stage-group (*, **)

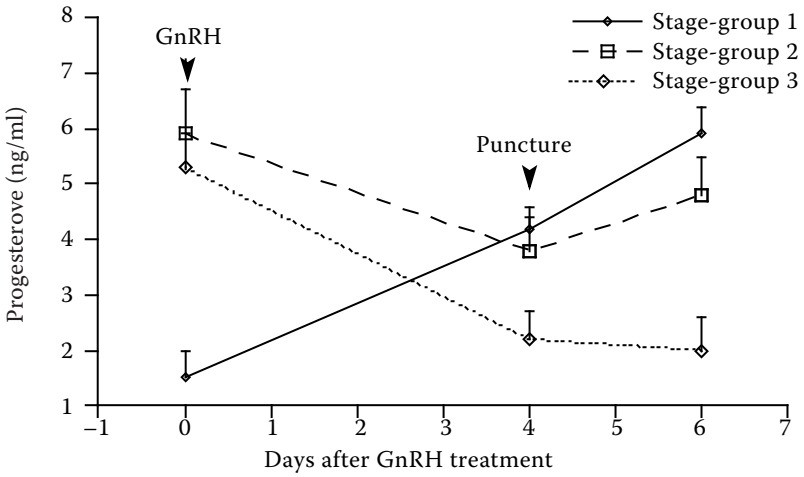

Figure 2. Mean progesterone concentrations on Days 0, 4 and 6 after GnRH treatment in three group heifers. GnRH was given between Days 0 and 7 (Stage-group 1; n = 15), 8 and 12 (Stage-group 2; n = 8) or 13 and 20 (Stage-group 3; n = 13) of the oestrous cycle (oestrus = Day 0); four days after GnRH treatment (Day 4), the follicles ≥ 6 mm were punctured (n = 21) or left intact (n = 15)

tion. Data within punctured (Stage 1, n = 7; Stage 2, n = 6; Stage 3, n = 8) and nonpunctured (Stage 1, n = 8; Stage 2, n = 2; Stage 3, n = 5) groups on Days 4 (follicular puncture), 6 (initiation of superstimulation), 10 (oestrus), and 18 (embryo collection) were compared using Duncan's multiple-range test of SAS. For these analyses, the least squares means (± SEM) were used. Data were transformed to square root (X + 0.5) before analysis by the GLM procedure and Duncan's multiple-range test. The non-transformed values (X ± SEM) are reported here.

RESULTS

Changes in the follicular status and estradiol concentrations 4 days after GnRH treatment

The population of follicles ≥ 7 mm and 4 to 6 mm in diameter and the concentrations of estradiol differed (P < 0.05) among Stage-groups on day of GnRH treatment (Day 0; Figure 1A). Four days after GnRH treatment, the mean number of follicles ≥ 7 mm in diameter increased from 1.2 ± 0.1 to 1.8 ± 0.1 (P < 0.01) and no longer differed among Stage-groups (Figure 1A). The number of follicle 4 to 6 mm was higher (Figure 1B; P < 0.05) on Day 4 than on Day 0 if GnRH treatment was given during the late phase of the oestrous cycle (Stage-group 3), but not if given during earlier phases (Stage-groups 1 and 2) and the population of follicles 4 to 6 mm in diameter still differed (P < 0.01) among Stage-groups on Day 4. Concentrations of estradiol were lower (P < 0.05) on Day 4 than on Day 0 only if animals were treated with GnRH at the beginning of the oestrous cycle (Stage-group 1), but as for the population of follicles ≥ 7 mm, they no longer differed among Stage-groups on Day 4 (Figure 1C; P > 0.1).

Progesterone concentrations increased from 1.5 ± 0.5 to 5.9 ± 0.5 ng/ml in heifers treated with GnRH between Days 0 and 7 (Stage-group 1), but they did not change (Stage-group × Day; P < 0.001; Figure 2) in those treated between Days 8 and 12 (Stage-group 2) of the oestrous cycle (5 to 6 ng/ml). Progesterone concentrations in heifers of Stage-group 3 which ranged from 5 to 6 ng/ml decreased after GnRH treatment, but the levels measured 4 and 6 days after GnRH treatment did not decrease below 1 ng/ml and averaged approximately 2 ng/ml (Figure 2).

The formation of an accessory CL occurred in 5/15, 4/8, 4/13 of heifers treated with GnRH between Days 0 and 7 (Stage-group 1), Days 8 and 12 (Stage-group 2), and Days 13 and 20 (Stage-group 3) of the oestrous cycle, respectively. Spontaneous oestrus never occurred prior to cloprostenol-induced luteolysis in heifers treated with GnRH in Stages 1, 2 or 3 of the oestrous cycle.

Changes in the follicular status and estradiol concentrations in response to follicular puncture

As expected, the mean number of follicles ≥ 7 mm in diameter on the day of the initiation of superstimulation (Day 6) was lower in heifers of the punctured group than in those of the nonpunctured group (Figure 1a; 0.19 ± 0.1 vs. 1.53 ± 0.1; P < 0.01). Similarly, concentrations of estradiol were lower on Day 6 in heifers of the GnRH-punc-

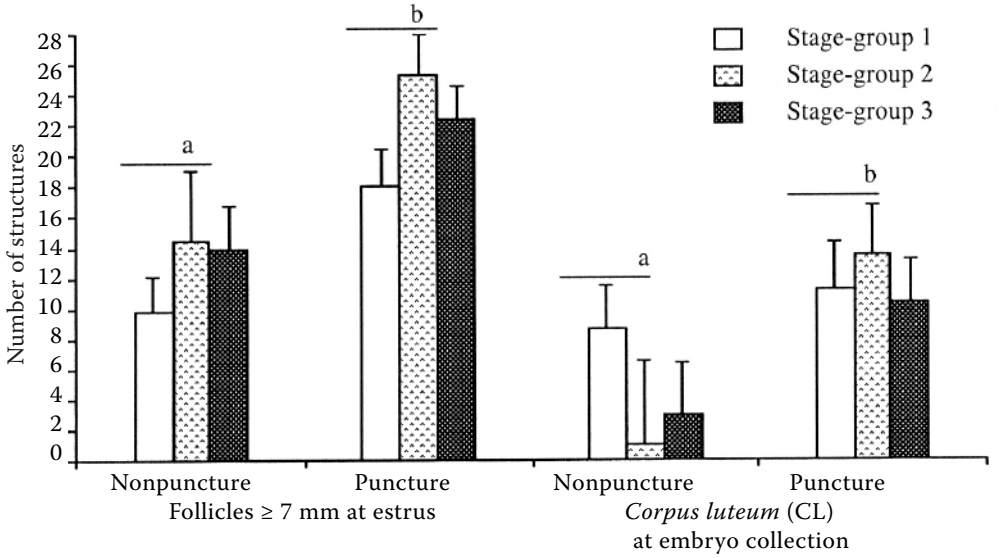

Figure 3. The mean number of follicles ≥ 7 mm in diameter at oestrus (Day 10 after GnRH treatment) and of corpora lutea (CL, by ultrasonography) at embryo collection (Day 18 after GnRH treatment) in three groups of heifers; GnRH was given between Days 0 and 7 (Stage-group 1; n = 15), 8 and 12 (Stage-group 2; n = 8) or 13 and 20 (Stage-group 3; n = 13) of the oestrous cycle (oestrus = Day 0); four days after GnRH treatment (Day 4), the follicles ≥ 7 mm were punctured (n = 21) or left intact (n = 15); all heifers were superstimulated with FSH from Days 6 to 10 after GnRH treatment; different superscripts on the bars (a, b) indicate a significant difference (P < 0.05) between punctured and nonpunctured groups

tured groups than in those of the GnRH-nonpunctured group (Figure 1C; 3.72 ± 0.6 vs. 7.17 ± 0.7; P < 0.001). The mean number of follicles 4 to 6 mm in diameter decreased (Figure 1B; 5.1 ± 0.8 vs. 2.9 ± 0.8) between Days 4 and 6 after GnRH treatment in heifers of the nonpunctured group but increased (Figure 1B; 6.8 ± 0.7 vs. 9.6 ± 0.6) in heifers of the punctured group (Stage-group × Day; P < 0.01). The population of follicles ≥ 7 mm and 4 to 6 mm as well as the concentrations of estradiol on Day 6 (Figure 1) were similar (P > 0.1) among Stage-groups within the punctured group, but not within the nonpunctured group (P < 0.05).

Influence of follicular puncture on superovulatory responses

Following the superstimulation with FSH, the mean number of follicles ≥ 7 mm in diameter at oestrus was higher (Figure 3; 21.8 ± 1.4 vs. 11.9 ± 1.7; P < 0.01) in heifers of the punctured group than in those of the nonpunctured group, but estradiol concentrations did not differ (Figure 4; P > 0.1) between the two groups. The number of CL at the time of embryo collection in the punctured group was similar to that of heifers of the control group superstimulated in a conventional manner

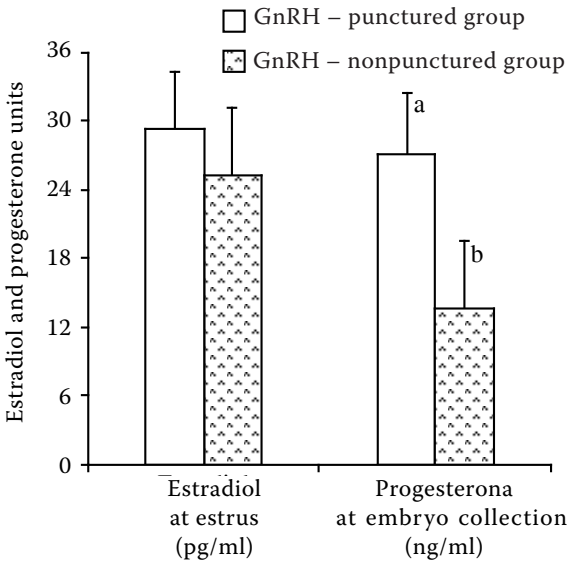

Figure 4. Estradiol (at oestrus; Day 10) and progesterone (at embryo collection; Day 18) concentrations following the GnRH treatment and follicular puncture; GnRH was given between Days 0 and 7 (Stage-group 1; n = 15), 8 and 12 (Stage-group 2; n = 8) or 13 and 20 (Stage-group 3; n = 13) of the oestrous cycle (oestrus = Day 0); four days after GnRH treatment (Day 4), the follicles ≥ 6 mm were punctured (n = 21) or left intact (n = 15); all heifers were superstimulated with FSH from Days 6 to 10 after GnRH treatment; different superscripts on the bars (a, b) indicate a significant difference (P < 0.05) between punctured and nonpunctured groups

($P > 0.1$) and higher than in heifers of the nonpunctured group (10.5 ± 1.5 and 12.5 ± 1.5 vs. 5.4 ± 2.1; $P < 0.01$). Similarly, progesterone concentrations on the day of embryo collection (Day 18) were higher (Figure 4; $P < 0.05$) in heifers of the punctured group compared to those of the nonpunctured group. These superovulatory responses were not affected ($P > 0.1$) by the stage of the oestrous cycle when GnRH treatment was given.

The *in vivo* production of transferable embryos was similar in the punctured and nonpunctured group ($P > 0.1$) and was considerably lower in these two groups than in the control group superstimulated in a conventional manner (1.6 ± 0.9 and 0.4 ± 1.3 vs. 6.5 ± 0.8, $P < 0.01$). The low embryo production in the punctured and nonpunctured groups in this experiment was associated with a high number of unovulated follicles (8.0 ± 1.2 vs. 7.6 ± 1.7, $P > 0.1$) that were still present on the day of embryo collection (Day 18). The punctured group was characterized by abnormally large ovaries with a very high number of follicles at oestrus. In general, cloprostenol-induced oestrus in GnRH treated heifers tended to occur 12 h sooner than in control untreated heifers (approximately 36 h vs. 48 h).

DISCUSSION

Treatment with GnRH was used in the present experiment to synchronize follicular waves at any stage of the oestrous cycle and to expose a dominant follicle for puncture at a predictable time. Since the animals were at various stages of the oestrous cycle, the population of follicles differed widely among Stage-groups when GnRH was given. Nevertheless, the homogeneity of follicular inventories occurred prior to superstimulation, but depending on the classes of follicles (i.e. large follicles ≥ 7 mm or recruitable follicles 4 to 6 mm) being considered, differences in the time interval needed to reach homogeneity were observed among Stage-groups. While different on Day 0, the number of follicles ≥ 7 mm and estradiol concentrations which mostly originate from these large follicles (Ireland et al., 1984; Bridges and Fortune, 2003; Fortune et al., 2004) were similar among Stage-groups 4 days after GnRH treatment.

In contrast to larger follicles, the population of recruitable follicles was still heterogeneous among Stage-groups, 4 days after GnRH treatment. An earlier study (Kohram et al., 1998b) showed that the number of recruitable follicles 4 to 6 mm increased following the GnRH treatment and that this is indicative of the emergence of a new follicular wave within 3 to 4 days. This suggests that the rate of entry of recruited follicles during a GnRH-induced wave emergence varies according to the stage of the oestrous cycle when GnRH was given. The heterogeneity of this population of follicles persisted among Stage-groups 2 days later (i.e. on Day 6) in nonpunctured animals. However, as observed previously, the puncture of all follicles ≥ 6 mm 4 days after GnRH treatment led to a significant increase in the number of recruitable follicles 2 days later (Kohram et al., 1998b) and on the day of initiation of superstimulation, this population of recruitable follicles no longer differed among Stage-groups. Generally, these results suggest that as a result of the combined use of GnRH and follicular puncture, the increased follicular turnover may lead to optimal ovarian conditions for superstimulation, i.e. absence of a dominant follicle and a high and homogeneous population of recruitable follicles (Bungartz and Niemann, 1994; Baracaldo et al., 2000; Lima et al., 2007).

As expected, the follicular puncture performed 4 days after GnRH treatment had no effect on progesterone concentrations. The present results confirm our earlier claim (Kohram et al., 1998b) that GnRH prevents the occurrence of spontaneous oestrus until cloprostenol-induced luteolysis during superstimulation with FSH, particularly in animals in the late phase of the oestrous cycle when treatment is initiated. Higher follicular (number of follicles ≥ 7 mm at oestrus) and ovulatory (number of CL and progesterone concentrations at embryo collection) responses to superstimulation with FSH are also observed when puncture is performed after GnRH treatment than when large follicles are left intact (Kohram et al., 1998a; Kim et al., 2001). Furthermore, such responses were observed whatever progesterone concentrations or stage of the oestrous cycles were at the time of GnRH treatment. When compared to animals superovulated in a conventional manner between Days 8 and 12 of the oestrous cycle (presumably under optimal conditions), ovulatory responses were similar in GnRH-punctured animals treated at any stage of the oestrous cycle.

However, despite these high follicular and ovulatory responses, striking differences in embryo production were observed between treated heifers and those that were superstimulated in a conventional manner (control). Wolfsdrof et al. (1997) reported that the follicular puncture 2 days before superstimulation increased the follicular response

at oestrus and number of ovulations at embryo collection, while the number of embryos did not differ between groups of punctured and nonpunctured control heifers. Production of embryos in the first experiment was considerably lower in both GnRH-treated groups of heifers than in control heifers (1.6 ± 0.9 and 0.4 ± 1.3 vs. 6.5 ± 0.8; $P < 0.01$) and this occurred whether the puncture was performed or not after GnRH treatment. Such a drastic decrease contrasts with data based on limited observations indicating that the embryo production following follicular synchronization with GnRH and superstimulation in cows appeared to be normal (Kohram et al., 1998a). Causes of such reduction are still unknown, but they may be associated with the abnormally high occurrence of anovulatory follicles and/or with an overstimulation of the ovarian system, particularly in GnRH-punctured heifers. It has been shown that ovarian overstimulation leads to a large increase in the number of anovulatory follicles (Saumande and Chupin, 1986; Guilbault et al., 1987).

In summary, results from the present study indicate that regardless of the stage of the oestrous cycle, the homogeneity of follicular inventories following the follicular synchronization is obtained within 4 days of GnRH treatment for follicles ≥ 7 mm but that follicular puncture is further needed to reach homogeneity within the population of recruitable follicles 4 to 6 mm before the initiation of superstimulation treatment. Although apparently normal in a prior study with cows (Kohram et al., 1998a), the *in vivo* production of embryos was compromised in the present study with heifers following the follicular synchronization with GnRH. This procedure is advantageous for the *in vivo* and *in vitro* production of bovine embryos since the spontaneous oestrus is eliminated, the procedure could be initiated at an unknown stage of the oestrous cycle without need for detection of the ovarian status at the initiation of superstimulation, and high follicular and ovulatory responses are obtained. However, more investigations are needed to increase the competence of oocytes obtained following this procedure.

REFERENCES

Andrade J.C., Oliveira M.A., Lima P.F., Santos A.S., Pina V.M. (2002): Use of steroid hormone treatments prior to superovulation in Nelore donors. Animal Reproduction Science, 69, 9–14.

Baracaldo M.I., Martinez M.F., Adams G.P., Mapletoft R.J. (2000): Superovulatory response following transvaginal follicle ablation in cattle. Theriogenology, 53, 1239–1250.

Bridges P.J., Fortune J.E. (2003): Characteristics of developing prolonged dominant follicles in cattle. Domestic Animal Endocrinology, 25, 199–214.

Bungartz L., Niemann H. (1994): Assessment of the presence of a dominant follicle and selection of dairy cows suitable for superovulation by a single ultrasound examination. Journal of Reproduction and Fertility, 101, 583–591.

Fortune J.E., Rivera G.M., Yang M.Y. (2004): Follicular development: the role of the follicular microenvironment in selection of the dominant follicle. Animal Reproduction Science, 82–83, 109–126.

Greve T., Callesen H., Hyttel P., Hoier R., Assey R. (1995): The effects of exogenous gonadotropins on oocyte and embryo quality in cattle. Theriogenology, 43, 41–50.

Guilbault L.A., Roy G.L., Brassard P., Ennaji A. (1987): Superovulation by continuous aortic infusion of follicle stimulation hormone (FSH-P) in cattle. Theriogenology, 27, 233 (Abstract).

Guilbault L.A., Roy G.L., Grasso F., Matton P. (1988): Influence of pregnancy on onset of oestrus and luteal function after prostaglandin-induced luteolysis in cattle. Journal of Reproduction and Fertility, 84, 461–468.

Guilbault L.A., Grasso F., Lussier J.G., Rouillier P., Matton P. (1991): Decreased superovulatory responses in heifers superovulated in the presence of a dominant follicle. Journal of Reproduction and Fertility, 91, 81–89.

Huhtinen M., Rainio V., Aalto J., Bredbacka P., Makitanila A. (1992): Increased ovarian responses in the absence of a dominant follicle in superovulated cows. Theriogenology, 37, 457–463.

Ireland J.J., Fogwell R.L., Oxender W.D., Ames K., Cowley J.L. (1984): Production of estradiol by each ovary during the estrous cycle of cows. Journal of Animal Science, 59, 764–771.

Kim H., Son D.S., Yeon S.H., Choi S.H., Park S.B., Ryu I.S., Suh G.H., Lee D.W., Lee C.S., Lee J.H., Yoon J.T. (2001): Effect of dominant follicle removal before superstimulation on follicular growth, ovulation and embryo production in Holstein cows. Theriogenology, 55, 937–945.

Kohram H., Bousquet D., Durocher J., Guilbault L.A. (1998a): Alteration of follicular dynamics and superovulatory responses by gonadotropin releasing hormone and follicular puncture in cattle: a field trail. Theriogenology, 49, 1165–1174.

Kohram H., Twagiramungu H., Bousquet D., Durocher J., Guilbault L.A. (1998b): Ovarian superstimulation

after follicular wave synchronization with GnRH at two different stages of the estrous cycle in cattle. Theriogenology, 49, 1175–1186.

Lima W.M., Vieira A.D., Thaller Neto A., Mezzalira A., Matos R.C., Gregory R.M. (2007): Improved superovulatory response in beef cattle following ovarian follicular ablation using a simplified transvaginal device. Animal Reproduction Science, 100, 364–370.

Pieterse M.C., Vos P.L., Kruip T.A., Wurth Y.A., Beneden T.H.V., Willemse A.H., Taverne A.M. (1991): Transvaginal ultrasound guided follicular aspiration of bovine oocytes. Theriogenology, 35, 857–862.

Romero A., Albert J., Brink Z., Seidel G.E. (1991): Numbers of small follicles in ovaries affect superovulatory response in Holstein cows treated with FSH-P in different endocrine states. Theriogenology, 35, 915–929.

SAS (1985): User's Guide: Statistics. SAS Institute, Inc., Cary, NC, USA.

Saumande J., Chupin D. (1986): Induction of superovulation in cyclic heifers: the inhibitory effect of large doses of PMSG. Theriogenology, 24, 233–247.

Twagiramungu H., Guilbault L.A., Dufour J.J. (1995): Synchronization of ovarian follicular waves with a gonadotropin-releasing hormone agonist to increase the precision of estrus in cattle: a review. Journal of Animal Science, 73, 3141–3150.

Wehrman M.E., Fike K.E., Kojima F.N., Bergfeld E.G., Cupp A.S., Mariscal V., Sanchez T., Kinder J.E. (1996): Development of persistent ovarian follicles during synchronization of estrus influences the superovulatory response to FSH treatment in cattle. Theriogenology, 45, 593–610.

Wolfsdrof K.E., Diaz T., Schmitt E.J., Thatcher M.J., Drost M., Thatcher W.W. (1997): The dominant follicle exerts an interovarian inhibition on FSH-induced follicular development. Theriogenology, 48, 435–447.

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