and brought to laboratory in saline solution on ice (0–1°C). Spermatozoa were collected by aspirating the sperm-rich fluid from convoluted tubules beneath the ductus deferens with a sterile hypodermic needle attached to a 10-mL syringe containing 2–4 mL of TRIS-tes egg yolk diluent and stored overnight at 4°C. The sperm suspension was aliquoted into 3 tubes, washed twice with fertilization medium (TALP; Parrish *et al*. 1985 Theriogenology 24, 537); pellets were dissolved in 1 mL of fertilization medium supplemented with 10 μg/mL heparin or 10 μM/mL Ca-I or no additive (control). Aliquots (200 μL) were made in Eppendorf tubes and incubated at 38°C for 0.5, 1, 1.5, or 2 h. Sperm viability and acrosomal status was evaluated by a dual staining procedure (Didion *et al*. 1989 Gamete Research 22, 51–57). Briefly, spermatozoa were incubated with an equal volume of 0.2% trypan blue for 10 min and washed twice (centrifugation at 700*g* for 6 min) with fertilization medium. Smears were made on glass slides and dried quickly on a warm stage. Slides were stained with 10% giemsa stain for 40 min. They were rinsed under a stream of distilled water, air-dried, and covered with DPX mountant and coverslips. The experiment was replicated six times. Spermatozoa ($n > 200$/slide) were examined in randomly selected microscopic fields under a phase-contrast microscope at 1000×. Spermatozoa were classified as dead (stained blue in the post-acrosomal region), live non-acrosome-reacted (unstained post acrosomal region but light purple-dark pink acrosome) or live acrosome reacted (unstained acrosome and post-acrosomal region). Data were analyzed using Student's *t*-test and are presented as mean percent ± SEM. There was no difference in the proportion of live acrosome-reacted (LAR) and live non-acrosome-reacted (LNAR) spermatozoa in the heparin group compared to the control group (Table 1). Although Ca-I group had higher LAR sperm, the proportion of dead spermatozoa was high ($P < 0.05$). It may be concluded that heparin was not able to enhance the acrosome reaction, and Ca-I seems to be detrimental to the spermatozoa at this concentration. Further investigations are warranted to evaluate the appropriate concentrations and duration of exposure to both of these agents for induction of acrosomal reaction in spermatozoa of this species.

Table 1

Treatment	Time (h)	LAR	LNAR	Dead
Heparin (10 μg/mL)	0.5	8.8 ± 1.8	55.2 ± 1.9	36.0 ± 1.9
	1	10.4 ± 1.4	55.0 ± 4.4	34.6 ± 4.3
	1.5	9.8 ± 1.2	55.5 ± 3.7	34.7 ± 2.5
	2	9.4 ± 1.5	54.7 ± 8.1	35.9 ± 6.5
Calcium ionophore (10 μM/mL)	0.5	18.4 ± 4.7*	16.5 ± 3.8†	65.1 ± 5.7*
	1	16.7 ± 3.3*	5.4 ± 1.3†	77.9 ± 2.3*
	1.5	18.3 ± 3.0*	2.5 ± 1.5†	79.1 ± 1.7*
	2	12.8 ± 3.8	2.2 ± 1.3†	85.0 ± 3.6*
Control	0.5	7.3 ± 1.0	58.2 ± 4.3	34.5 ± 4.6
	1	8.7 ± 1.0	51.6 ± 4.9	39.7 ± 3.8
	1.5	10.7 ± 1.7	51.4 ± 4.4	37.8 ± 3.1
	2	9.5 ± 1.6	50.9 ± 6.2	39.6 ± 5.8

* Significantly higher; † significantly lower than other values in same column, Student's *t*-test ($P < 0.05$).

Folliculogenesis/Oogenesis

202 GENETIC INFLUENCE ON FOLLICULAR DEVELOPMENT IN CATTLE

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In cattle, the development of ovarian follicles >5 mm occurs in waves. During each estrus cycle, a cohort of 5–7 follicles develops either 2 or 3 times, with the last wave containing the ovulatory follicle. It is speculated that follicular wave number within estrus cycle may be genetically determined; however, no data have been published to support this hypothesis. The present study was designed to test this hypothesis by using genetically identical cloned cows and comparing their wave patterns among each other and to those of control animals by studying their ovarian follicular dynamics. Three 5-yr-old cloned cows, derived from somatic cells of a 13-yr-old Holstein dairy cow, were age- and lactational status-matched with contemporary cows produced by AI, and maintained under the same managemental conditions. Ovarian follicular dynamics of all the cows were determined using a real-time, B-mode, linear-array ultrasound scanner equipped with a 7.5 MHz transrectal transducer (Aloka Co., Tokyo, Japan). Ultrasound examinations were carried out every day for 101 days and covered 4 estrus cycles for each animal. For tracking the follicular dynamics, daily ultrasound observations were videotaped and individual follicle dimension and location were drawn. The estrus was determined by the visual observation of a growing follicle over a period of several days, reaching a maximum diameter of about 15 mm or more, and its subsequent disappearance on the following day. The ultrasound observations were corroborated with the visual and behavioral signs of estrus. The average length of estrus cycle in clones and controls was 23.08 ± 0.47 days and 22.67 ± 0.37 days (mean ± SEM), respectively, and were not significantly different. Out of the four estrus cycles studied, all clones showed 3 follicular waves in 3 cycles and 2 waves in one cycle. However, the wave pattern within each cycle did not match for the clones over all the cycles. In controls, one cow had 2 waves in all 4 cycles while the other two cows had both 3- as well as

2-wave cycles. The 3-wave cycles had an average length of 23.89 ± 0.59 days (mean ± SEM) and 24.00 ± 0.71 days in clones and controls, and the average lengths of 2-wave cycles were 20.67 ± 0.33 d and 22.00 ± 0.38 d in clones and controls, respectively. There was no significant difference in the average cycle lengths among the three clones, which were 23.00 ± 1.23, 23.75 ± 1.38, and 22.50 ± 0.65 days. Over the 4 estrus cycles, the clones and the controls had a total of 60.33 ± 7.45 and 63.00 ± 6.12 small (4 mm–<6 mm), 63.74 ± 6.34 and 69.00 ± 9.62 medium (6 mm–10 mm), and 22.00 ± 2.51 and 20.00 ± 1.16 large (>10 mm) follicles, respectively. The total number of follicles for each clone during 4 cycles were different, *viz*., large (25, 17, 24), medium (60, 76, 55), and small (71, 64, 46). Thus the clones, in spite of their shared genetic makeup, had different cycle lengths, follicle numbers, and follicular wave patterns. This indicates that genetic makeup is not the only major influence determining bovine follicular dynamics.

203 ASSESSMENT OF OVARIAN RESERVE. IS THERE A ROLE FOR OVARIAN BIOPSY?

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The pool of primordial follicles in the ovary or ovarian reserve is a major factor in human fertility potential. In bovine medicine as well, this ovarian reserve has been linked to the results of superovulation procedures (Cushman *et al*. 1999 Biol. Reprod. 60, 349–354). These authors suggested a biopsy to assess the level of this reserve. Whether the biopsy(ies) is(are) a true reflection of the follicular distribution in the ovarian cortex, is (to the best of our knowledge) a factor never investigated until now in bovine medicine. In human medicine, this procedure has been critically examined for that particular use and found not to be suited (Lass *et al*. 2004 Hum. Reprod. 19, 467–469). Indeed, randomized or "blind" sampling of one biopsy is adequate only if follicles are evenly spread in the ovarian cortex; in any case they are not deeper than a few mm from the surface. Moreover, the quantitative counting of follicles does not provide any information about the quality of the oocytes embedded in them. Taking a biopsy of a bovine ovary in a minimally invasive way is technically feasible (Aerts 2004 Reprod. Fertil. Dev. 16, 229–230). Therefore, the aim of this study was to examine the natural distribution of primordial follicles in the ovarian cortex of bovine ovaries. Slaugtherhouse ovaries were collected at random. The volume (mL) was measured and the macroscopically visible follicles were counted. Then the ovaries were cut in slices of 5 Âμm, and every 8th ($8 \times 5 = 40$ μm interval) slice was subjected to fixation in formalin and hematoxylin-eosin staining. Before counting of the primordial follicles, the ovarian cortex was subdivided into 8 equal parts. These "parts" were supposed to mimick a (single) ovarian biopsy. The 8 parts of a slice represent here multiple biopsies. For each of these parts, the number of primordial follicles was counted; only follicles with a visible oocyte were included. The results of the parts containing the ligament of the ovary were excluded. Results are shown in Table 1. The results show that the distribution of primordial follicles between small parts of the bovine ovarian tissue was extremely uneven. A large variation was observed between samples obtained from the same ovary. Moreover, an extrapolation of follicle numbers found in biopsies to entire ovaries were hampered by the uneven size and morphology of these ovaries. Therefore, we conclude that the use of single biopsies of ovarian cortex for a quantitative evaluation of the ovarian reserve has limited value; an empty cortex or a cortex with very few follicles might be just incidental and meaningless. Even the use of multiple biopsies, although less variable, does not solve the problem of extrapolation of these data to entire ovaries.

Table 1. Macroscopically visible follicles on 4 ovaries and primordial ("microscopical") follicles on 4 slices of each of these ovaries

Ovary	Volume	Total follicles			
		Macro (ovary)	Micro (4 slices)	Follicles/slice mean ± SD (range)	Follicles/part* mean ± SD (range)
1	12	17	198	49.5 ± 15.5 (31–69)	6.78 ± 3.66 (2–20)
2	5	9	247	61.8 ± 14.6 (49–81)	8.50 ± 3.79 (3–17)
3	6	1	83	20.8 ± 4.8 (15–26)	3.33 ± 1.99 (0–8)
4	19	10	242	60.5 ± 6.8 (52–68)	8.28 ± 4.33 (0–16)

* A "part" = a slice divided by 8, and mimicks an ovarian biopsy.

204 ASSOCIATION BETWEEN SOME ULTRASONIC CHARACTERISTICS OF CORPUS LUTEUM AND PROGESTERONE VALUES IN CASPIAN MINIATURE MARES

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Reproductive characteristics of the Caspian miniature horse have some similarities to other equine breeds, but several differences such as follicular wave pattern, multiple ovulations, and the size of ovulatory follicle have been reported. Still there are some other characteristics to be determined. This study was carried out to investigate ultrasonic and hormonal changes of the Caspian mares during estrus cycle. Eleven clinically healthy and cycling Caspian mares, aged 3–17 years and weighing 159–236 kg L.W., were used from April to July. Using an ultrasound scanner equipped with a 5-MHz, B-mode, linear array rectal transducer, formation of the corpus luteum was observed from Day 0 (day of ovulation), and changes in plasma progesterone were investigated by daily blood collection and assay by means of an RIA method. Repeated measure ANOVA test was used

for statistical analysis. The maximum size of the corpus luteum was seen at Day 3 postovulation (36.6 ± 1.91 mm), and remained up to 10 days after ovulation. At Days 10–11 a significant decrease in size was observed; thereafter gradual regression occurred and minimum size was reached by Days 16–17 (17.3 ± 1.46 and 16.9 ± 1.20 mm, respectively). The first significant increase in progesterone level was detected 24 h after ovulation and average high values of 8 ng/mL by Day 4 after ovulation. The average peak value of progesterone was detected on Days 7 to 10 of the cycle (11.6 ± 1.37 ng/mL) and remained so until Day 12. The lowest plasma progesterone concentration (less than 1 ng/mL) was observed from Day 14 till one day postovulation, coinciding with the CL regression found in sonographic observations. The results of this study revealed that the pattern of corpus luteum formation and Its changes in size and the plasma progesterone levels during the estrus cycle in this breed follow a pattern similar to that of other equine breeds.

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205 DEVELOPMENT OF OOCYTES FROM COWS TREATED WITH RETINOL IS COMPROMISED PRIOR TO IMPLANTATION

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In the cow, sheep, and gilt, a retinoid support enhances the oocyte development (reviewed by Hidalgo C *et al*. 2003 Reproduction 125, 409–416). The shortest effective time interval for these animals injected with retinol (ROH) is four days, but the reasons for this have not been described. Furthermore, the development after transfer of embryos derived from oocytes of donors treated with ROH is not known. The objective of the present work was to elucidate the two above questions. We analyzed the ROH and retinyl palmitate (REP) contents in plasma and follicular fluid (FF). The estrus cycles of heifers ($n = 12$) were synchronized with progestagen and PGF2α. Blood samples were taken at progestagen removal (Day 0). Animals were injected with 1×10^6 ROH units ($n = 6$) or corn oil (vehicle; $n = 6$) on Days 0, 1, and 4. Contents of follicles between 3 and 10 mm were aspirated on Day 4 by an ultrasound-guided procedure. All samples were analyzed for ROH and REP by HPLC. To produce embryos for transfer, follicles were aspirated from donors treated four times with 1×10^6 ROH units/week or vehicle, starting four days before the first aspiration. Both groups of cumulus-oocyte complexes (COCs) were matured *in vitro* with or without 5 nM 9-cis-retinoic acid (RA). The presence of RA prolonged the exposure to retinoids (cows treated with ROH) or acted as a positive control (heifers with vehicle). Oocytes were fertilized and cultured in mSOF + 5% FCS. Embryo transfer (ET) to recipients was performed with fresh (one) or vitrified (two) good-morphology Day 7 embryos, and pregnancy monitored on Days 21, 35, and 60. Data analysis was by GLM (ROH and REP concentrations) or CATMOD (pregnancy monitoring), and Duncan's test ($^{a,b}P < 0.05$; $^{v,x,y,z}P < 0.02$). Average FF volume recovered were 351 ± 127 μL (controls; range 100–700) and 393 ± 127 μL (ROH; range 80–950). Concentrations of REP were unaffected by timing, follicle or blood, and ROH treatment (data not shown). Concentrations of ROH (μg/dL) for vehicle and ROH-treated cows were (LSM ± SE) 42.0 ± 1.8^{vx} and 42.0 ± 2.4^{vx} (plasma-Day 0, before ROH injection), 37.3 ± 1.8^{v} and 47.64 ± 2.4^{x} (plasma-Day 1), 42.6 ± 1.9^{vx} and 45.5 ± 2.3^{vx} (plasma-Day 4), and 6.1 ± 3.0^{y} and 16.8 ± 2.6^{z} (FF-day 4), respectively. Cumulatively, embryos from donors receiving ROH did not exhibit pregnancy on Day 21 ($0/15^{x}$; confirmed on Day 35), which differed from vehicle donors on Day 21 (44%, $8/18^{y}$) and Day 35 (33%, $6/18^{y}$), and tended to differ ($P = 0.07$) on Day 60 (22%, 4/18). Pregnancy rates were independent of fresh or vitrified embryos within ETs. Injected ROH elevated blood concentrations of ROH, although values became normalized on Day 4. However, a coasting period of 4 days for ROH administration seems to be justified by increased intrafollicular levels of ROH. Development into blastocysts is disrupted before implantation, showing that ROH directly affects the oocyte during its intrafollicular growth.

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206 FOLLICULAR GROWTH SUBSEQUENT TO FOLLICULAR ASPIRATION IN THE MARE

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Follicle aspiration has previously been used in mares as a research tool to remove growing and atretic follicles, in order to study follicular growth. The aim of the present study was to (1) evaluate the fate of the aspirated follicles, and (2) determine the point of selection of the dominant follicle subsequent to follicular aspiration. In six Standardbred mares, all follicles larger than 9 mm were removed by transvaginal ultrasound-guided aspiration (Day 0). Subsequent to follicular aspiration, the growth of follicles larger than 1 mm was monitored daily by ultrasonography from Day 0 to Day 7. Aspirated follicles were monitored to establish whether they refilled and continued to grow, or luteinized. The experiment was conducted in six replicates in each mare. On Day 1 after aspiration the largest and second largest follicles were 10.4 ± 0.8 mm (mean ± SEM) and 7.8 ± 0.6 mm, respectively. On Day 7 the largest follicle and the second largest follicle were 25.7 ± 1.2 mm and 18.6 ± 1.2 mm, respectively. In 10/209 follicles, the follicular cavity refilled subsequent to aspiration with non-echogenic fluid and the follicle diameter increased during the following 7 days. Four of the ten aspirated and refilled follicles grew to become the largest follicles, whereas the remaining six follicles did not reach dominance. A further three aspirated follicles grew and ovulated on Day 5. In one case, an aspirated follicle refilled and continued to grow after an oocyte had been recovered. However, from these preliminary results, the growth pattern of the aspirated follicle can not be predicted on the basis of whether or not the oocyte was removed during the aspiration session. Preliminary results of this study indicate that follicular selection for dominance as determined by follicular size difference may already have occurred on Day 1 after aspiration. Furthermore, follicles that refill with fluid and continue to grow after aspiration may pose a problem when follicular growth and selection are studied.

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207 OOCYTE SURVIVAL AND FOLLICULAR DEVELOPMENT IN Fas-KNOCKOUT AND KIT-DEFICIENT DOUBLE MUTANT MICE

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Growth factors and cytokines regulate survival and growth of mammalian oocytes via their cognate receptors. Among those receptors, KIT, a receptor tyrosine kinase, has been thought of as an essential molecule for growth and survival of oocytes and for follicular development. The defect of KIT-mediated signals leads to the loss of oocytes and impairment of follicular development. Fas is a member of the death receptor family inducing apoptosis; it expresses in the ovary. In a previous study (Sakata *et al*. 2003 Cell Death Differ. 10, 676–86), we generated KIT-deficient and Fas-knockout double mutant (W^v/W^v:$Fas^{-/-}$) mice to study the relation between Fas and KIT signaling in germ cell apoptosis. To further understand the role of KIT in oocyte survival and follicular development, we examined the ovaries of W^v/W^v and W^v/W^v:$Fas^{-/-}$ in comparison to those of C57BL/6 (wild type) mice. We also examined the possibility of overcoming the deleterious effects of KIT deficiency by ovarian allotransplantation. One ovary of each mouse was fixed for immediate histological examination and the other was transplanted under the kidney capsule of a female SCID (severe combined immune deficiency) mouse. Ovaries and recovered grafts were fixed, embedded, serially sectioned at 5 μm, stained with hematoxylin and eosin, and examined under a microscope. Oocytes were counted in every section where the nucleus was seen, avoiding double counting in adjacent sections. Mean (with standard deviation) numbers of oocytes per graft or ovary were compared using Student's *t*-test. At 13 days post-coitum (dpc), ovaries of W^v/W^v fetuses contained 1104.3 ± 118.8 ($n = 4$) germ cells which was significantly ($P < 0.05$) lower than those of wild-type mice. However, at 16 dpc ($n = 6$) and 2-days old ($n = 6$), ovaries did not contain any germ cells/oocytes. After allotransplantation of the ovaries ($n = 6$) from W^v/W^v fetuses (13 dpc) for 2 weeks, all of the germ cells disappeared. When the ovaries from 2-day-old W^v/W^v mice ($n = 6$) were allotransplanted for 12 days, no oocytes appeared. On the other hand, transplanted ovaries from C57BL/6 fetuses (13 dpc) contained 2162.0 ± 97.3 ($n = 6$) oocytes after 2 weeks. In those ovaries, 4.7 ± 1.6% follicles developed to secondary follicles which contained growing oocytes. Importantly, ovaries of 2-day-old W^v/W^v:$Fas^{-/-}$ mice ($n = 4$) contained 1936.0 ± 245.0 oocytes (64.0 ± 10.0% of wild-type mice), and 14-day-old mice ($n = 4$) still contained 911.3 ± 106.3 follicles in which 28.6 ± 6.0% and 11.4 ± 3.2% follicles developed to primary and secondary follicles, respectively. These results indicate that oocyte death due to KIT-deficiency can not be rescued by ovarian transplantation in SCID mice, and that the Fas-knockout condition partially prevents the death of oocytes induced by KIT-deficiency, and primordial follicles develop in this condition.

208 FOLLICULAR DYNAMICS, ESTRUS, AND OVULATION IN COWS SYNCHRONIZED TO OVULATE FIRST OR SECOND WAVE DOMINANT FOLLICLES USING SHORT- OR LONG-TERM MELENGESTROL ACETATE- OR EAZI-BREED CIDR INSERT-BASED PROTOCOLS

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The objective of this experiment was to determine the feasibility in substituting EAZI-BREED CIDR inserts (CIDR; Pfizer Animal Health, Groton, CT, USA) for melengestrol acetate (MGA) in progestin-based protocols to synchronize estrus in beef cows. Follicular dynamics, timing of estrus, and ovulation were compared in beef cows synchronized to ovulate first or second wave dominant follicles using short- or long-term MGA- or CIDR-based protocols. The study was conducted with 48 nonsuckled, estrous cycling, crossbred beef cows assigned to one of four treatments (T1 to T4; $n = 12$/T) by age and body condition. Cows were synchronized to ovulate first wave (T1 and T2) or second wave (T3 and T4) dominant follicles based on assignment to treatment. Cows in T1 were fed MGA ($0.5\ mg\ h^{-1}\ d^{-1}$) for 7 days, and were injected with $PGF_{2\alpha}$ (PG; 25 mg Lutalyse; Pharmacia Animal Health, Kalamazoo, MI, USA) on Day 7, GnRH (100 μg Cystorelin; Merial, Athens, GA, USA) on Day 11, and PG on Day 18. Cows in T2 had CIDR (1.38 g progesterone) inserted for 7 days, and were injected with PG on Day 7, GnRH on Day 9, and PG on Day 16. Cows in T3 were fed MGA for 14 days, and were injected with GnRH on Day 26, and PG on Day 33. Cows in T4 had CIDR inserted for 14 days, and were injected the GnRH on Day 23, and PG on Day 30. Transrectal ultrasonography was performed daily to monitor follicular dynamics from GnRH to estrus after PG; and every 4 h from 20 h after the onset of estrus until ovulation. Estrus detection was performed continuously using the HeatWatch® estrus detection system (DDx, Denver, CO, USA). Blood samples for progesterone (P_4) were collected daily beginning one day prior to the initiation of treatment and continuing through ovulation following PG. Data were analyzed using the General Linear Models procedure of SAS (SAS Institute, Inc., Cary, NC, USA) and are summarized in the following table. Animals that responded to treatment and were included in the analysis were those that initiated a new follicular wave after administration of GnRH and that displayed estrus within 144 h after PG. Although estrous response was similar among treatments, there were differences in follicular dynamics, steroid secretion patterns, and timing of events that culminated in differences in timing and synchrony of estrus and ovulation among the short- and long-term groups. These differences may be important in relation to fixed-time AI programs. These data suggest that in situations that are not conducive to feeding MGA, substituting CIDR inserts into MGA-based protocols to synchronize estrus may be feasible.

	Sample time	1st wave		2nd wave	
		T1 $n=11$	T2 $n=9$	T3 $n=11$	T4 $n=9$
P_4 (ng/mL)	At GnRH	0.1 ± 0.3^{a}	0.4 ± 0.3^{a}	2.7 ± 0.3^{b}	1.9 ± 0.3^{b}
	At PG	1.8 ± 0.4^{a}	1.7 ± 0.4^{a}	3.7 ± 0.4^{b}	3.9 ± 0.4^{b}
Follicle diam. (mm)	At PG	13.0 ± 0.5^{a}	12.9 ± 0.5^{a}	12.2 ± 0.5^{a}	10.7 ± 0.5^{b}
	At 20 h	15.2 ± 0.4^{ab}	15.3 ± 0.4^{ab}	15.7 ± 0.4^{a}	14.4 ± 0.4^{b}
PG to estrus (h)	Mean	51 ± 2.1^{a}	52 ± 2.4^{a}	62 ± 2.1^{b}	59 ± 2.4^{b}
	Range	$41–65^{a}$	$42–66^{a}$	$47–71^{a}$	$54–65^{b}$
Estrus to ovulation (h)	Mean	31 ± 1.2^{a}	27 ± 1.4^{b}	30 ± 1.2^{ab}	30 ± 1.4^{ab}
	Range	$26–46^{a}$	$22–38^{ab}$	$26–34^{c}$	$26–34^{bc}$

[abc] Unlike superscripts within a row differ, $P < 0.05$.

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209 NORGESTOMET IMPLANTS REDUCE LH RELEASE PATTERN IN ZEBU COWS UNDERGOING REPEATED OOCYTE PICK-UP

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Ultrasound-guided follicular puncture (OPU) has become the most used technique to recover cumulus-oocyte complexes (COCs) from valuable donors for *in vitro* embryo production, because of the low risk and the possibility of collecting COCs at intervals as short as twice-a-week. However, repeated aspiration of ovarian follicles may induce endocrine abnormalities due to partial luteinization of punctured follicles and interference with follicular development. The use of exogenous progestagens is an alternative used to control these side effects, and is under evaluation. The aim of this study was to evaluate whether the effect of norgestomet treatment on intra-follicular and systemic steroid concentrations and on ovarian follicular dynamics is related to changes in LH releasing pattern. Pluriparous non-lactating Gir breed (*Bos indicus*) cows ($n = 10$) were randomly distributed between treatment (norgestomet ear implants, replaced weekly) and control (no hormone used) groups, and had their ovarian follicles larger than 3 mm in diameter aspirated twice a week, during the next two consecutive weeks. Follicular dynamics were evaluated every 12 h between OPU sessions, and the largest follicles present were used to recover samples of follicular fluid. Blood samples were collected daily for progesterone evaluation in all cows, and 3 times within a 4-h window interval, at 24, 48, 72, and 96 h after follicle puncture for LH evaluation, in 6 cows (3 from treated and 3 from control groups). LH was measured by a standardized RIA procedure. Data was analyzed by ANOVA, and means were compared by Tukey's test. Results are presented as means ± SEM. Treatment with norgestomet reduced mean progesterone plasma concentration during the evaluated period (36.3 ± 14.0 vs. 250.3 ± 49.3 pg/mL; $P < 0.0001$), the incidence of follicles growing above 9 mm (30% vs. 65%; $P < 0.05$) in the intervals between OPU sessions, and intrafollicular estradiol and progesterone concentrations in the largest follicles ($n = 27$) present (265.5 ± 47.4 and 34.9 ± 5.4 ng/mL vs. 765.2 ± 169.1 and 173.3 ± 43.4 ng/mL, respectively; $P < 0.05$). Plasma LH concentrations were consistently lower during the 3 session intervals in cows treated with norgestomet (0.16 ± 0.04, 0.22 ± 0.03, 0.22 ± 0.09 and 0.17 ± 0.01 vs. 0.44 ± 0.15, 0.53 ± 0.04, 0.42 ± 0.05 and 0.39 ± 0.11 for 24, 48, 72, and 96 h after OPU, respectively; $P < 0.05$). These results confirm the theory that norgestomet treatment is associated with a reduction in the LH-release pattern, as expected due to the reduction in both luteinization of punctured follicles and in the steroidogenic activity of growing follicles observed during the experiment. The use of norgestomet ear implants can be an alternative in the management of donor cows undergoing oocyte pickup.

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Gene Expression

210 COMPARISON OF REAL-TIME PCR AND END-POINT PCR FOR ANALYSIS OF GENE EXPRESSION IN PREIMPLANTATION EMBRYOS

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The purpose of this study was to compare real-time PCR and end-point PCR with respect to their suitability for the analysis of gene expression in samples in which the number of cells is limited, for example, in studies of pre-implantation embryonic development. The real-time instrument was a