

EFFECT OF TRANSVAGINAL ULTRASOUND GUIDED PUNCTURE OF FOLLICLES ON CYCLICITY IN HEIFERS

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Ultrasound guided transvaginal follicular puncture has been developed (Pieterse et al., 1988, Pieterse et al., 1991) as a method to obtain oocytes for in vitro fertilization from live animals. Follicles can be punctured at all stages of the estrous cycle and consecutively during several months (Pieterse et al., 1988, Pieterse et al., 1991, Bergfelt et al., 1994, Kruip et al., 1994, Vos et al., 1994, Paul et al., 1995, Bungartz et al., 1995). Follicular development is a dynamic process throughout the estrous cycle and associated with cyclicity of animals. As have been reported by Pieterse et al. (1988) aspiration of the larger follicles in cows early in the follicular phase delayed the start of luteal phase and aspiration of these follicles at the end of the estrous cycle increased the cycle length (Pieterse et al., 1991). In the present experiment the influence of transvaginal puncture of follicles on cyclicity in heifers were investigated.

Material and Methods

Two heifers of the Swedish Red and White breed (13 and 14 months of age) were subjected to follicular puncture once a week. For puncture of follicles a special stainless steel holder equipped inside with endovaginal multiple angle transducer (Pie Medical, 5.0-7.5 MHz) and on ventral position with single lumen needle guide was used. All follicles ≥ 3 mm were punctured and the follicular fluid was aspirated. The cyclicity of heifers was determined on the basis of estrous signs and progesterone concentrations in blood plasma. The plasma progesterone concentrations were determined using enhanced luminescence immunoassay procedure. The intra-assay coefficient of variation was below 4% between 2 and 160 nmol/l. The mean inter-assay coefficient of variation was below 15.3 % for three quality control samples (1.6, 17.5 and 46.05 nmol/l). The detection limit of the assay was 0.18 nmol/l.

Results and Discussion

In total, 18 aspiration sessions were performed during 7 estrous cycles. Three puncture sessions per cycle were performed during 4 and two puncture sessions per cycle during 3 estrous cycles. The lengths of the estrous cycles varied from 19 to 21 days, with a mean length of 19.71 ± 0.28 days, which is regarded to be a normal cycle length in non-aspirated heifers in agreement with observations by Kastelic et al. (1990). The mean length of estrous cycles in which two or three puncture sessions were performed was not significantly different, 19.33 ± 0.33 and 20.0 ± 0.40 days, respectively ($P > 0.05$). On an average, the P_4 levels reached maximum concentrations (21.73 ± 3.78 and 25.0 ± 0.25 nmol/l) at days 10.30 ± 1.66 and 10.25 ± 0.05 ($P > 0.05$) of the estrous cycle, depending on number of puncture sessions per cycle. There was a slight, but non-significant difference in the mean length of the luteal phases (interval of estrous cycle between progesterone levels ≥ 1.0 nmol/l) with two (13.33 ± 1.20 days) and three (16.25 ± 0.47 days) aspiration sessions per cycle ($P > 0.05$) and related to the reported patterns of estrous cycles (Ginther et al., 1989, Stubbings & Walton, 1995).

After aspiration of the large follicles (≥ 10 mm) at estrus (one time) or 2-4 days before estrus (three times) formed luteal structures were observed by ultrasonography in the ovaries and increase of plasma progesterone levels on day 2 and 3 of subsequent estrous cycles. Similar findings have been reported elsewhere, but it was not determined, however, if the luteal structures resulted from luteinization of the ablated follicles or from undetected ovulations (Bergfelt et al., 1994). Also, after aspirations of oocytes twice a week in FSH stimulated cows formation of luteal structures were observed by Stubbings & Walton (1995). In another study, using unstimulated cows, luteal structures were formed, but no indications at increased progesterone levels were seen (Gibbons et al., 1994). Aspiration of follicular contents does not obliterate the follicular wall and may leave behind viable granulosa cells. Increased pulses of LH observed during the preovulatory period as well may likely play a role (Spiser & Echterkamp, 1986, Pierson & Ginther, 1987, Bergfelt et al., 1994, Stubbings & Walton, 1995).

Conclusions

We can conclude that once a week repeated punctures of follicles during a period of several estrous cycles have no influence on cyclicity in heifers. It would be useful to develop further critical studies to investigate and confirm the luteinization of punctured follicles.

References

- Bergfelt DR, Lightfoot KC, Adams GP: Ovarian synchronization following ultrasound guided transvaginal follicle ablation in heifers. *Theriogenology* 1994; 42: 895-907.
- Bungartz L, Lucas-Hahn A, Rath D, Niemann H: Collecting of oocytes from cattle via follicular aspiration aided by ultrasound with or without gonadotropin pretreatment and in different reproductive stages. *Theriogenology* 1995;43: 667-675.
- Edmondson AJ, Fissore RA, Pashen RL, Bondurant RH: The use of ultrasonography for the study of the bovine reproductive tract. I. Normal and pathological ovarian structures. *Anim Reprod Sci* 1986;12:157-176.
- Gibbons JR, Beal WE, Krisher RL, Faber EG, Pearson RE, Gwazdauskas FC: Effects of one- versus twice weekly transvaginal follicular aspiration on bovine oocyte recovery and embryo development. *Theriogenology* 1994;42:505-419.
- Ginther OJ, Knopf L, Kastelic JP: Temporal associations among ovarian events in cattle during oestrous cycles with two and three follicular waves. *J Reprod Fert* 1989; 87:223-230.
- Kastelic JP, Bergfelt DR, Ginther OJ: Relationship between ultrasonic assesment of the corpus luteum and plasma progesterone concentration in heifers. *Theriogenology* 1990;33:1269-1278.
- Kruip ThAM, Boni R, Wurth YA, Roelofsen MWM, Pieterse MC: Potential use of ovum pick-up for embryo production. *Theriogenology* 1994;42:675-684.
- Paul JB, Looney CR, Lindsay BR, Godke RA: Gonadotropin stimulation of cattle donors at estrus for transvaginal oocyte collection. *Theriogenology* 1995;43: 294 (abstr).
- Pierson RA, Ginther OJ: Follicular populations during estrous cycle in heifers. I. Influence of day. *Anim Reprod Sci* 1987;14:165-176.
- Pieterse MC, Kappen KA, Kruip ThAM, Taverne MAM: Aspiration of bovine oocytes during transvaginal ultrasound scanning of the ovaries. *Theriogenology* 1988;30:752-762.
- Pieterse MC, Vos PLAM, Kruip ThHM, Willemsse AH, Taverne MAM: Characteristics of bovine estrous cycles during repeated transvaginal, ultrasound-guided puncturing of follicles for ovum pick-up. *Theriogenology* 1991;35:401- 413.
- Spiser LJ, Echternkamp SE: Ovarian follicular growth, function and turnover in cattle: a review. *J Anim Sci* 1986;62:428-451.
- Stubbings RB, Walton JS: Effect of ultrasonically guided follicle aspiration on estrous cycle and follicular dynamics in Holstein cows. *Theriogenology* 1995;43:705-712.

Folliikulite korduva, ultraheli kontrolli all toimuva transvaginaalse punkteerimise mõju mullikate innatsüklile

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Kokkuvõte

Kahel mullikal teostati 7 innatsükli jooksul kokku 18 transvaginaalset ovotsüütide aspireerimisprotseduuri. Kahel innatsükliil tehti kaks ja neljal innatsükliil kolm aspireerimisprotseduuri. Hormonaalse analüüsi tulemused näitasid, et folliikulite punkteerimine ovotsüütide aspireerimiseks üks kord nädalas ei mõjutanud mullikate innatsükli. Innatsükliite pikkus varieerus 19 kuni 21 päevani ja ei sõltunud sellest, kas ovotsüüte aspireeriti innatsükli jooksul kaks või kolm korda, olles keskmiselt vastavalt 19,33 ja 20,0 päeva ($P < 0,05$). Uuringu põhjal võib järeldada, et ultraheli kontrolli all teostatud korduv transvaginaalne antraalsete folliikulite punkteerimine ovotsüütide saamiseks *in vitro* viljastamise ja kultiveerimise eesmärgil ei mõjuta mullikate innatsükli pikkust. Edaspidi on vajalik uurida punkteeritud folliikulites toimuvaid muutusi.