First Follicular Wave Pattern of Kedah-Kelantan Cows Stimulated with Different Types of Gonadotropin Hormone Regimes for Transvaginal Oocyte Aspiration

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Abstract

The aim of the study was to compare the first wave pattern of follicular development in terms of mean number of follices and follicular size of Kedah-Kelantan beef cows stimulated with four different protocols of gonadotropin hormone regime. Twenty four Kedah-Kelantan (KK) beef cows were treated with four different regimes: A) Follicle Stimulating Hormone (FSH) administered a total of 18 NIH units in a dividing dose at decreased volume twice a day for 3 consecutive days beginning on day 4 of controlled internal drug release (CIDR®) insertion, B) FSH, administered a total of 18 NIH units in a dividing dose at decreased volume twice a day for 4 consecutive days begining on day 4 of CIDR® insertion, C) Pregnant Mares' Serum Gonadotropin administered 2500 iu intramuscularly on day 4 of CIDR® insertion, and D) FSH injected a total of 18 NIH units intramuscularly in a dividing dose at decreased volume twice a day for 4 consecutive days beginning on day 9 of prostaglandin injection. Ovaries were visualized with a 7.5 MHz linear array transrectal transducer attached to a portable ultrasound scanner. In regimes A and B, a higher number of follicles was available on days 1 and 2, and on days 0 to day 2, respectively. However, the medium size follicles of regimeB and C were mostly available in greater number on day 1, and day 1 to day 2, respectively. Similarly in regime C, availability of higher number of medium follicles was on day 1 to day 2, small follicles on day 0, and total number of follicles was in greater number on day 2. While in regime D, a greater number of follicles was available on day 2. The results showed that regimes A and B had significantly higher ($P<0.05$) total number of follicles compared to C and D. The trend of follicular development with various types of gonadotropin hormone regimes was similar for all regimes with small follicles were in greater number at the early part of the growth phase and subsequently followed by an increase in medium and larger follicles. As a conclusion OPU could be done on day 1 for regimes A and B and day 2 for regimes C and D, or the whole duration of OPU could be conducted from day 1 to 3 after CIDR® removal or the last injection of PGF for all four gonadotropin hormone regimes.

Keywords: CIDR®, beef cows, follicular wave, gonadotropin hormone

Introduction

Attempts to produce genetically superior donors through *in vitro* production of embryos have been reported in many parts of the world (Beal *et al*., 1992; Getz *et al*., 2000; Galli *et al*., 2001). Most studies have utilized ovaries recovered from slaughtered cows to yield oocytes used in *in vitro* embryo production of embryos. However, oocytes of this origin are usually of poor quality and of unknown genetic background (Stangl *et al*., 1999). Alternatively, oocytes are harvested from live oocyte donors, and this is where an understanding of follicular growth and development plays a critical role to ensure quality oocytes are obtained. Therefore, techniques have been developed to repeatedly harvest oocytes from live donors (Broadbent *et al*., 1997). Techniques such as laparotomy and laparoscopy were carried out on small size animals such as calves (Armstrong *et al*., 1992; Armstrong *et al*., 1994) and lambs (Armstrong *et al*., 1994; Berlingeur *et al*., 2003). In large animals such as cattle, buffalo and horse, transvaginal ultrasound-guided aspiration or ovum pick-up (OPU) technique has been applied (Armstrong *et al*., 1994; Azizah *et al*., 2000). The ovarian physiology such as follicular dynamic activity (Mapletoft *et al*., 2005) which included the study on follicle number and development capacity (Damiani *et al*., 1995; Duby *et al*., 1995) should be understood well before the technique could be applied. Follicular dynamics is evaluated using visual data analysis through ultrasonography images. In cattle, antral follicles of 3-8 mm in diameter are the main suppliers of oocytes used for embryo production by OPU. This population shows a high dynamics due to the occurrence of follicular waves. Follicular wave is a process where a cohort of antral follicles begin to mature and grow under the influence of sufficient pituitary gonadotropic stimulation to permit progression towards ovulation (Rajakoski, 1960). Individual follicles can be divided into three phases: growth, plateau and regressive in a series of follicular development. The period from emergence of a wave to apparent cessation of progressive growth was defined as the growth phase. Plateau phase is the period from cessation of growth from the apparent progressive decrease in diameter. Regressive phase can be defined as the period following the end of the plateau phase (Fortune *et al*., 1988). It had been reported that the developmental competence of oocytes was affected by the stages of the follicular wave but the effect of different stages of the follicular wave on the developmental competence of the oocytes was difficult to predict (Hendriksen *et al*., 2004). Therefore, effort has to be made to test the developmental competence of OPU-collected oocytes on the three different stages of the follicular waves.

The study of follicular development requires standard protocols for synchronisation and induction with exogenous gonadotropin in order to develop a proper timing to conduct OPU. Gonadotropin has been widely used to stimulate and induce follicular development (Lucy *et al*., 1992). Hence, the types of gonadotropin and dosage regimes are important factors in determining high follicle developmental competence and yield of quality oocyte. Many studies have been conducted to compare the oocyte quality of untreated animals and those which were treated with gonadotropin hormone (Stangl *et al*., 1999; Tervit *et al*., 1996). It had been shown that the multiple treatments with exogenous gonadotropin before follicular aspiration improved the number of quality oocytes recovered and increased embryo production of the processed oocytes (Berlingeur *et al*., 2003; Blondin *et al*.,

1996; Goodhand *et al*., 2000; Goodhand *et al*., 1999). Stangl *et al*. (1999) recovered a lower number of oocytes from untreated animals than from those stimulated with gonadotropin hormone. However, there was no significant difference found in the quality of recovered cumulus-oocyte complex. Therefore, due to the differences in the ability and efficacy of each gonadotropin, it is important to develop a standard protocol. These results can be adopted to optimise the OPU technique in order to improve the number and quality of oocytes for *in vitro* embryo production. Therefore, the objective of the study was to examine the follicular pattern of the first follicular wave development and mean number and size of follicles in Kedah-Kelantan (KK) cows stimulated with gonadotropin hormone.

Materials and Methods

Animals and Experimental Design

Twenty four KK cows aged 3 to 5 years old and weighing 370 to 450 kg were used in the present study. The cows were raised in a semi-intensive system with limited day grazing and night confinement. Cows were fed pellet feed containing 15.9 % crude protein and 17.6 MJ calculated gross energy (GE) once a day based on recommended maintenance energy requirement at a daily rate of one kg per 100 kg bodyweight. Cows were fed early in the morning for 14 days before scanning. Cows were divided into four regimes: A) Follicle Stimulating Hormone (FSH; Ovagen®, Immuno-chemical Products Ltd., New Zealand) administered intramuscularly in dividing doses of 5, 3 and 1 NIH unit at decreasing volume twice a day for 3 consecutive days beginning on a day 4 of controlled internal drug releasing device (CIDR-B®, InterAg, 558 Te Rapa Road, Hamilton, New Zealand) insertion, B) FSH, administered intramuscularly in dividing doses of 3.5, 2.5, 2 and 1 NIH unit at decreasing volume twice a day for 4 consecutive days beginning on a day 4 of CIDR® insertion, C) 2500 iu Pregnant Mares' Serum Gonadotropin (PMSG; Folligon®, Horizon Technology Pty Ltd) administered intramuscularly once on day 4 of CIDR® insertion, and D) FSH, injected intramuscularly in dividing doses of 3.5, 2.5, 2 and 1 NIH units at decreasing volume twice a day for 4 consecutive days beginning on day 9 of PGF injection. All cows in regimes A, B and C were at different stages of the oestrous cycle and were inserted with CIDR® containing 1.38 g progesterone intra-vaginally for 7 d and followed with 25 mg $PGF_2\alpha$ (Estrumate®; Schering-Plough Animal Health, Australia) injection intramuscularly two days prior to CIDR® removal before receiving hormonal treatment. CIDR® was removed on day 7 after insertion (day 0 equals to day of CIDR® removal or last injection of $PGF_{2\alpha}$). In regime D, cows were at different stages of the oestrous cycle and were injected with 3.0 NIH units of FSH at day 4 and again with 25 mg $PGF_2\alpha$ twice a day on day 12. Data on oestrus behaviour were recorded every 30 min for three days. Only cows that showed oestrous were selected for the follicular development study. The follicular mapping was carried out daily for 11 consecutive days. The study was conducted as a completely randomized design with 4 regimeregimes, each alloted with 6 cows. Response to regimeregimes was measured on follicular mapping on 11 consecutive daily readings.

Scanning Method

Ovaries were visualised with a 7.5 MHz linear array transrectal transducer (Aloka Co. Ltd, Japan) attached to a portable ultrasound (Aloka, Echo System,

SSD-500, Japan). The rectum was emptied from faeces and the positions of the ovaries were identified by rectal palpation. The transducer was lubricated and slowly introduced into the rectum. Each ovary was scanned several times in lateromedial and dorsoventral planes to determine the follicles. Follicles that were non-echogenic were delineated by an echogenic calliper at the interface of the follicular wall with ovarian stroma. The average follicle size was recorded based on the width of the largest and smallest shape measurement of follicle which was not spherical. Scanning was performed daily between 8 and 9 a.m. for 10 consecutive days after CIDR[@] removal. Size and relative dimension for both follicles and corpus luteum were sketched on follicle maps. Ovarian data were combined for the two ovaries of each animal. The follicles were divided into three categories as described by Alvarez *et al*. (2000) with slight modification: small (4 – 6 mm), medium (6.1 – 8.4 mm) and large (8.5 - > 10 mm)] categories.

Statistical Analyses

Mean size and total number of follicles were analysed with one-way analysis of variance (ANOVA) using SPSS version 19 statistical package. The differences between regimeregimes on follicular parameters were detected using Duncan's multiple range test for comparison of means. The relationship between days of oestrous cycle to the follicle size was analysed using Spearman's correlation. Significant differences were accepted at the probability of less than 0.05.

Results and Discussion

The super stimulation regime showed significant number of medium sizes (day 0 to 10; $P < 0.05$); however, there were no differences in mean large size ($P > 0.05$) and small size ($P > 0.05$; Table 1) follicles during the 10 days of oestrous cycles. In regime B, a higher number of follicles was available at day 0 to 2 ($P < 0.05$). The follicular mapping showed that medium size follicles were available in greater number on day 1 ($P < 0.05$; Table 2) and started to reduce starting from day 4 onwards. The results also showed that regime B produced a significantly higher mean total number of follicles ($P < 0.05$).

Super stimulation with FSH showed a significantly higher number of follicles was available at day 1 ($P < 0.05$, mean = 8) compared to days 2 to 10 (Table 2). The small size follicles were available in greater number from day 0 to 3, medium size follicles from day 1 to 2, and the large follicles from day 1 to 3 and from day 6 to 8.

Negative correlations between days of oestrous cycles and number of small size follicles ($P < 0.001$; $r = - 0.41$) and medium size follicles ($P < 0.0001$; $r = -0.44$) were also detected. The negative correlation existed between day of oestrous cycles and number of follicles of small ($P < 0.05$, $r = - 0.369$) and medium ($P < 0.05$, $r = - 0.52$) size, but did not correlate significantly with the large size follicles ($P > 0.05$).

Table 1: Mean number of various size follicles obtained from daily follicular mapping of the growth phase of first follicular wave development

Day	Gonadotropin hormone regimes*											
	A	B	C	D	A	B	C	D	A	B	C	D
	Small size**				Medium size**				Large size**			
0	3.00^{a}	2.33^{a}	2.67^{a}	1.17	0.83^{bc}	2.17^{ab}	1.33^{a}	1.17	0.83^{a}	2.83^{a}	1.33	1.83
1	2.67^{a}	3.33^{a}	1.17^{ab}	1.43	2.50^{a}	3.33^{a}	2.33^{a}	1.43	2.83^{a}	2.50^{a}	1.67	1.17
2	1.00^{c}	1.67^{a}	1.67^{ab}	1.5	2.33^{a}	1.83^{abc}	2.33^{a}	1.50	2.33^{a}	3.33^{a}	2.17	2.50
3	1.33^{abc}	2.00^{a}	2.00^{ab}	0.83	1.83^{abc}	2.17^{ab}	0.50^{c}	0.83	1.33^{a}	2.33^{a}	2.00	2.17
4	1.33^{bc}	1.17^{a}	0.50^{b}	0.83	1.33^{bc}	2.17^{ab}	2.00^{a}	0.83	1.33^{a}	2.50^{a}	1.50	1.43
5	1.33^{bc}	1.17^{a}	1.00^{ab}	0.83	1.33^{abc}	0.50^{bc}	1.17^{ab}	0.83	1.33^{a}	2.83^{a}	1.83	2.33
6	0.17^{c}	1.00^{a}	0.17^{b}	1.50	0.83^{bc}	0.67^{bc}	0.83^{b}	1.50	2.83^{a}	1.67^{a}	2.50	1.83
7	0.17^{c}	0.67^{a}	0.83^{b}	0.50	1.00^{bc}	1.17^{bc}	0.17^{c}	0.50	2.83^{a}	2.33^{a}	3.17	1.17
8	1.00^{c}	1.20^{a}	0.33^{b}	0.83	0.50^{c}	0.60^{bc}	0.33^{c}	0.83	2.50^{a}	2.20^{a}	2.17	1.17
9	1.17^{bc}	1.00^{a}	0.50^{b}	0.83	0.33^{c}	0.29^{c}	0.83^{b}	0.83	1.50^{a}	1.86^{a}	2.50	1.17
10	1.00^{c}	1.17^{a}	0.17^{b}	0.83	0.50^{c}	0.50^{bc}	0.50^{c}	0.33	2.17^{a}	1.67^{a}	3.17	1.17

^{abcd}Means within rows are significantly different ($P<0.05$)

* Gonadotropin hormone regimes of A, B, C and D

**Size of follicles: Small =4 – 6 mm, Medium = 6.1 –8.4 mm, Large = 8.5 - ≥ 10 mm

Table 2: Mean total number of follicles (mean ± SE) obtained from daily follicular scanning of the growth phase of follicular development

Day	Gonadotropin hormone regimes*			
	A	B	C	D
0	4.67 ± 0.558^{bc}	7.33 ± 0.95^{a}	5.33 ± 0.843^{ab}	5.33 ± 0.72^{a}
1	8.00 ± 1.653^{a}	9.17 ± 1.887^{a}	5.17 ± 0.703^{ab}	5.17 ± 0.96^{ab}
2	5.67 ± 0.558^{b}	6.83 ± 1.167^{abc}	6.17 ± 1.05^{a}	5.83 ± 0.48^{a}
3	5.17 ± 0.48^{bc}	6.50 ± 0.563^{abcd}	4.5 ± 0.76^{ab}	4.50 ± 0.56^{abc}
4	4.50 ± 0.56^{bc}	5.83 ± 0.79^{bcde}	4.0 ± 0.73^{abc}	4.17 ± 0.31^{abc}
5	4.00 ± 1.0^{bc}	4.50 ± 0.62^{bcde}	4.0 ± 0.68^{ab}	4.33 ± 0.80^{abc}
6	3.83 ± 0.54^{bc}	3.33 ± 0.49^{e}	3.5 ± 0.96^{bc}	4.33 ± 0.84^{abc}
7	4.00 ± 0.730^{bc}	4.17 ± 1.19^{cde}	4.17 ± 0.87^{ab}	2.50 ± 0.76^{c}
8	4.00 ± 0.577^{bc}	3.67 ± 0.76^{de}	2.83 ± 0.60^{bc}	2.67 ± 0.760^{c}
9	3.00 ± 0.68^{c}	3.17 ± 0.54^{e}	3.83 ± 0.70^{abc}	3.00 ± 0.58^{bc}
10	3.67 ± 0.61^{bc}	3.33 ± 0.84^{e}	1.50 ± 0.67^{c}	2.50 ± 0.67^{c}

^{abcde}Means between columns with different superscripts are significantly different ($P<0.05$)

* Gonadotropin hormone regimes of A, B, C and D

In PMSG' super stimulation (Regime C) showed significant difference in medium ($P < 0.05$) and small size follicle ($P<0.05$) categories of follicle numbers and sizes. Whereas there was no significant difference in mean large ($P > 0.05$) category of follicle. The negative correlation was also obtained between days of oestrous cycle with number of follicles of small ($P < 0.05$, $r = -0.43$) and medium ($P < 0.05$, $r = - 0.42$) size, but did not show similar correlation pattern with large size follicles ($P > 0.05$; $r = - 0.081$).

In regime D, superstimulation regime did not show any significant number of small ($P>0.05$), medium ($P > 0.05$) and larger ($P > 0.05$) sizes of follicles in ten days of oestrous cycle. However, the total number of follicles was significantly different ($P < 0.05$) in terms of producing follicles by the days, as indicated by a higher number of follicles on day 2. Significant correlation existed between days of oestrous cycle with small ($P < 0.05$; $r = -0.30$) and medium ($P < 0.05$; $r = -0.29$) sizes of follicles. However, the day of oestrous cycle did not correlate significantly with the number of large size follicles ($P > 0.05$).

The regimes were not significantly different in terms of number of small ($P > 0.05$) and medium ($P > 0.05$) size follicles. However, regimes had an effect on the number of large size follicles ($P < 0.05$). Among regimes, regime B had significantly higher number of large follicles (mean = 2.36, $P < 0.05$). In contrast, regime D showed significantly lower number of large follicles ($P < 0.05$, mean = 1.61). This gave an indication that the number of large follicles was influenced by the superstimulation regimes. Similarly, total number of follicles observed was significantly higher for regime B (5.25), followed by A (4.59), C (4.09) and D (4.03) regimes. Total number of follicles was significantly higher on day 1 than day 2 of the first follicular wave development.

Days of oestrous cycle had a significant effect on number of small ($P < 0.05$) and medium ($P < 0.05$) size follicles. However, days of oestrous cycle did not show significant difference in terms of the number of large size follicles ($P > 0.05$). Day 0 (2.58) and day 1 (2.46) had significantly higher mean number of small and medium size follicles, and day 2 (2.58) showed a higher number of large size follicles. There was a significant correlation between days of oestrous cycles and gonadotropin regimes ($P < 0.05$). The Spearman's correlation showed negative relationship existed between regimewith large size follicles ($P < 0.05$, $r = - 0.130$), and total follicle number ($P = 0.014$, $r = - 0.135$). Total follicle number of regimeB was available in greater number mostly at day 0 and day 2 ($P < 0.05$). The medium follicles were available in greater number on day 1 ($P < 0.05$). The correlation coefficients of day of oestrous cycle and treatments were - 0.38 ($P < 0.05$, $r = - 0.502$) and – 0.29 ($P < 0.05$, $r = - 0.135$), respectively.

The study on effect of gonadotropin on follicular development pattern was restricted from day 0 to day 10 of the oestrous cycle. In regimeA, a higher number of follicles was available on days 1 and 2 and regime B on day 0 to day 2. Medium size follicles of regime B were mostly available in greater number on day 1. Similarly in regime C, availability of higher number of medium follicles was on day 1 to day 2, small follicles on day 0, and total number of follicle was available in greater number on day 2. Similarly in regime D, a greater number of follicles was available on day 2. Overall, it could be suggested that OPU could be done on day 1, for regimes A and B; and day 2 for regimes C and D. Alternatively, the duration of OPU could be conducted from day 1 to 3 for all four types of gonadotropin hormone regimes.

Overall the gonadotropin hormone regimes evaluated gave a higher mean number of follicles at an earlier stage of the first wave of follicular development. Among the regimens, only B regime showed significantly ($P < 0.05$) higher mean total number of follicles starting on day 0 until day 5. Greater mean total number of follicles for regimes A, B, C and D were obtained from day 0 to day 3, day 0 to day 5, day 0 to day 3 and day 0 to day 3, respectively. The trends of follicular development regimes with various types of gonadotropin hormone regimes showed a similar pattern for all groups. It could be seen that small follicles were available in greater number at the early part of the growth phase and subsequently the reduction of the smaller ones was replaced by increase in the medium and larger follicles. An earlier study by Ireland *et al*. (1979) had shown that no heifer had follicles in the medium and large range per pair of ovaries during day 1 to day 4. However, in the present study more small follicles were observed on day 0 to day 1, medium follicles on day 1 to day 4 to day 6 and large follicle on day 2 to day 3, and day 7 to day 9. The availability of more follicles in those days would indicate that FSH had regulated the availability of medium and large follicles during day 1 to day 4.

A high dynamics due to the occurrence of follicular waves was also observed. It is suggested that the wave exists in such as a pattern on the three stages that are growing, beginning and late dominance, and regressing phases. Lucy *et al*. (1992) reported a cohort of small follicles (2 - 4 mm) was recruited out of the pool during an oestrus cycle. The follicles had increased in size to become medium size follicles (6 - 9 mm); and later single follicles were selected to continue to grow while others including the subordinate follicles decreased in size and became atretic follicles, whereas the increased number of small follicles on day 5 to day 9, would represent the loss of follicular dominance and the beginning of wave 2 of follicular development. The results showed that day 1 of regimes A and B showed significantly higher number of mean total follicle ($P < 0.05$) compared to C and D regimes. This suggests that the best time to do OPU would be day 1 due to the higher availability of mean total number of follicles and also day 1 was still negatively affected by the dominant follicles present up to the day of oestrus (Hendriken *et al*., 2004). During the first three days of follicular wave the FSH-responsive follicles would also grow. Then, from day 3 onwards, the future dominant follicle would start to grow faster than the follicles (Ginther *et al*., 1998) and keep on growing until about day 6. Whereas the smaller subordinate follicles would stop growing and become atretic. Dominant follicles would lose their dominance between days 8 and 10, leading to the onset of a new wave. The follicles of different stages of atresia were present at each stage of the follicular wave (Hagemann *et al*., 1999). However, the proportion of atretic follicles and degree of atresia decreased at the beginning and increased during the second half of the follicular wave (Hagemann *et al*., 1999).

The mean total number and size of small and medium categories of follicles were not significantly different ($P > 0.05$) between regimes. However, the total number of large category of follicles and size was significantly different ($P = 0.037$) between regimes. The mapping of the first wave of follicular development gave an indication of the mature characteristics of follicular fluids of ovary and environmental condition in the follicles (Iwata *et al*., 2006) which reflected the quality of oocytes. Thus, the total number of follicles recruited gave an indication of the response of individual

follicles to the pharmacologic agents used for ovarian super stimulation regimes.

In all regimes, it showed a similar pattern where days of oestrous cycle had effects with small ($P < 0.05$) and medium ($P < 0.05$) size follicles but had no effect on the larger size of follicle ($P > 0.05$). This would give an indication that the gonadotropin treatments had stimulating effects to increase the follicle size. In a follicular development, it was suggested that as a cohort of antral follicles developed, only one follicle was able to be dominant and suppressed the subordinate follicles from growing (Iwata *et al*. 2006). However in the present study, we were able to obtain more than one follicle of greater than 8.5 mm in size. An earlier study found that treating cows with FSH for stimulation subverted the selection process and created a cohort of dominant follicles (Mapletoft *et al*., 2005). Superovulated cows would lead the follicles in the cohort capable of maintaining dominance as FSH support was continued (Lucy *et al*., 2007). This would strengthen the suggestion that the hormonal regime affected more on the number of medium and large size follicles. The stimulation regimens therefore, could alter the number of medium to large follicles size and it could be adopted to obtain oocytes from follicles using OPU techniques.

The size of follicles was equally important because it reflected the activity of the follicles and only suitable size could be punctured, and aspirated through OPU technique. In general, follicles have to be highly atretic before the cumulus-oocyte-complexes show clear signs of degeneration and lack of developmental competence (Wurth *et al*., 1994; De Wit *et al*., 2000). In addition, the *in vitro* maturation, fertilization and culture of oocytes through OPU would be the best indicators of the most suitable and reliable hormone regime to produce embryos. The cost of each regime should be taken into consideration and determine the most reliable and optimal protocol for OPU.

Thus, gonadotropin enhanced the number of larger follicles at early stages of oestrous cycle and increased the size of these follicles. Therefore, the present study suggests that the OPU could be done on day 1 and day 2 after CIDR® removal or the last injection of PGF, for regimes A and B; and regimes C and D, respectively. Alternatively, the duration of OPU could best be done up to day 3 with any of the superstimulation hormone regimes evaluated in the present study.

Conclusion

The hormonal regime was shown to have an effect on the available number of medium and large size follicles by altering the number of small to medium and large size follicles, and it could be adopted to obtain oocytes from follicles using OPU techniques. Besides giving an indication on the selection of pharmacologic agents used for ovarian super stimulation regimes, super stimulation effect of gonadotropin also showed the importance of gonadotropin hormones for *in vivo* development and growth of ovarian follicles suitable for OPU. The duration of OPU could be conducted in the range from days 1 to 3 after CIDR® removal or the last injection of PGF for the four types of gonadotropin hormone regimes.

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References

Alvarez, P., Spicer, L.J., Chase, C.C., Payton, Jr., M.E., Hamilton, T.D. and Stewart, R.E. 2000. Ovarian and endocrine characteristics during an oestrous cycle in Angus, Brahman, and Senepol in a subtropical environment. J Anim. Sci. 78: 1291-1302.

Azizah, A., Sharif, H., Zawawi, I., Mohd Khusahry, M.Y., Krishnalingam, M., Shapii, S.M., Tasol, S., Md Salleh, S., Zainuddin, H., Norham, H., Ramli K. and Razli M. A. 2000. Proc. 22nd Malaysian Society of Animal Production Annual Conference, Kota Kinabalu, Sabah, Malaysia; 85-86.

Armstrong, D.T., Irvine, B.J., Earl, C.R., McLean, D. and Seamark, R.F. 1994. Gonadotropin stimulation regimes for follicular aspiration and *in vitro* production from calf oocytes. Theriogenology 427: 1227-1236.

Armstrong, D.T., Holm, P., Irvine, B.J., Petersen, B.A., Stubbing, R.B., McLean, D. ,Stevens, G. and Seamark, R.F. 1992. Pregnancies and live birth from *in vitro* fertilization of calf oocytes collected by laparoscopic follicular aspiration. Theriogenology 38: 667-678.

Beal, W.E., Perry, R.C. and Corah, L. 1992. The use of ultrasound in monitoring reproductive physiology of beef cattle. J. Anim. Sci. 70: 924-929.

Berlingeur, F.G., Leoni, G., Bogliolo, L., Pintus, P.P., Rosati, I., Ledda S. and Naittana, S. 2003. FSH different regimes affect the development capacity and cyotolerance of embryos derived from oocyes collected by ovum pick-up in dopor sheep. Theriogenology 61: 1477-1486.

Blondin, P., Coenen, K., Guibault, L. A. and Sirard, M.A. 1996. Superovulation can reduce the developmental competence of bovine embryos. Theriogenology 46: 191-1203.

Broadbent, P.J., Dolman, D,F., Watt, R.G., Smith, A.K. and M.F. Franklin, M.F. 1997. Effect of the frequency of follicle aspiration on oocyte yield and subsequent superovulatory response in cattle. Theriogenology 47**:** 1027-1040.

Damiani, P., Fissore, R.A., Cibelle, J.B., Robl, J.B. and Duby, R.T. 1995. Evaluation of cytoplasmic maturation of calves oocytes. Theriogenology 43:191 abstract.

De Wit, A.A., Wurth, Y.A. and Kruip, T.A. 2000. Effect of ovarian phase and follicle quality on morphology and developmental capacity of the bovine cumulus–oocyte complex. J. Anim. Sci. 78: 1277–1283.

Duby, R.T., Damiani, P., Looney, C.R., Long, C. R., Blaise, J. J. and Blaise, J. M.. 1995. Cytological characterization of maturation and fertilization in prepubertal calf oocytes. Theriogenology 43: 202 abstract.

Fortune, J.E., Sirois, J. and Quirk, S.M. 1988. The growth and differentiation of ovarian follicles during the bovine estrous cycle. Theriogenology 29(1): 95-109.

Galli, C., Crotti, G., Nottari, C., Turini, P., Duchi, R. and Lazzari, G. 2001. Embryo production by ovum pick up from live donors. Theriogenology 55: 1341-1357.

Getz, I., Matkovic, M., Lojkić, M., Makek, Z., Baćić, G. and Cergolj, M. 2000. Transvaginal ovum pick-up in the cow: alternative in the production of bovine embryos for transfer. Vet. arhiv 70 (Suppl.). S75-S80.

Ginther, O.J., Bergfelt, D.R., Kulick, L.J. and Kot, K. 1998. Pulsatility of systemic FSH and LH concentration during follicular-wave development in cattle. Theriogenology 50: 507-519.

Goodhand, K.L., Stainess, M.E., Hutchinson, J.S.M. and Broadbent, P.J. 2000. *In vivo* oocyte recovery and *in vitro* embryo production from bovine oocyte donors treated with progestagen, oestradiol and FSH. Anim. Reprod. Sci. 63: 145-158.

Goodhand, K.L., Watt, R.G., Stainess, M.E., Hutchinson, J.S.M. and Broadbent, P.J. 1999. *In vivo* oocyte recovery and *in vitro* embryo production from bovine donors aspirated at different frequencies for following FSH treatment. Theriogenology 51: 951-961.

Hagemann, L., Beaumont, S., Berg, M., Donnison, M., Ledgard, A. and Peterson, A. 1999. Development during IVP of bovine oocytes from dissected follicles: interactive effects of estrous cycle stage, follicle size and atresia. Mole. Reprod. Dev. 53: 451–458.

Hendriksen, P.J.M., Steenweg, W.N.B., Harkema, J.C., Merton, J.S., Bevers, M.M., Vos, P.L.A.M. and Dielman, S.J. 2004. Effect of different stages of the follicular wave on in vitro developmental competence of bovine oocytes. Theriogenology 61: 909-920.

Ireland, J.J., Coluson, P.B. and Murphree, R.L. 1979. Follicular development during four stages of the estrous cycle of beef cattle. J. Anim. Sci. 49: 1261-1269.

Iwata, H., Inoue, J., Kimura, K., Kuwayama, T. and Monji, Y. 2006. Comparison between the characteristics of follicular fluid and the developmental competence of bovine oocytes. Anim. Reprod. Sci. 91: 215-23.

Lucy, M.C. 2007. The bovine dominant ovarian follicle. Retrieved on February 23, 2009, from www.asas.org/cgi/content/full/5/13_suppl/E89. J. Anim. Sci. 5:E89-E99.

Lucy, M.C., Savio, J.D., Badinga, L., De La Sota, R.L. and. Thatcher, W.W. 1992. Factors that affect ovarian follicular dynamics in cattle. J. Anim. Sci. 70: 3615-3626.

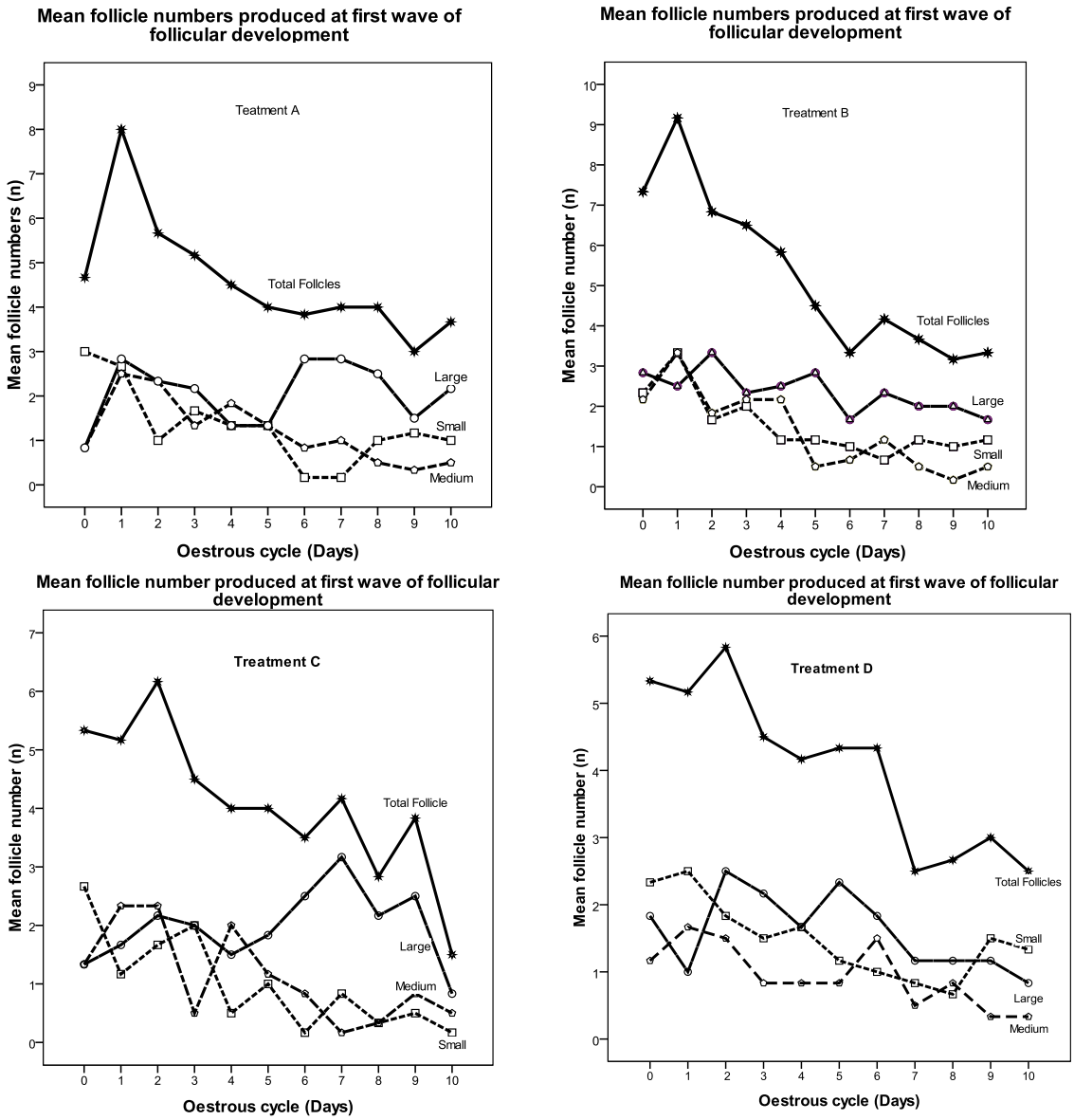
Mapletoft, R.J. and Hasler, J.F. 2005. Assisted reproductive technologies in cattle. A review. Rev. Sci. Technol. 24: 393-403.

Rajakoski, E. 1960. The ovarian system in sexually mature heifers with special reference to seasonal, cyclical, and left-right variations. Acta Endocrinology Copenhagen Supplement. 52: 7-68.

Stangl, M., Kuhholzer, B., Besenfelder, U. and Brenn, G. 1999. Repeated endoscopic ovum pick-up in sheep. Theriogenology 52: 709-716.

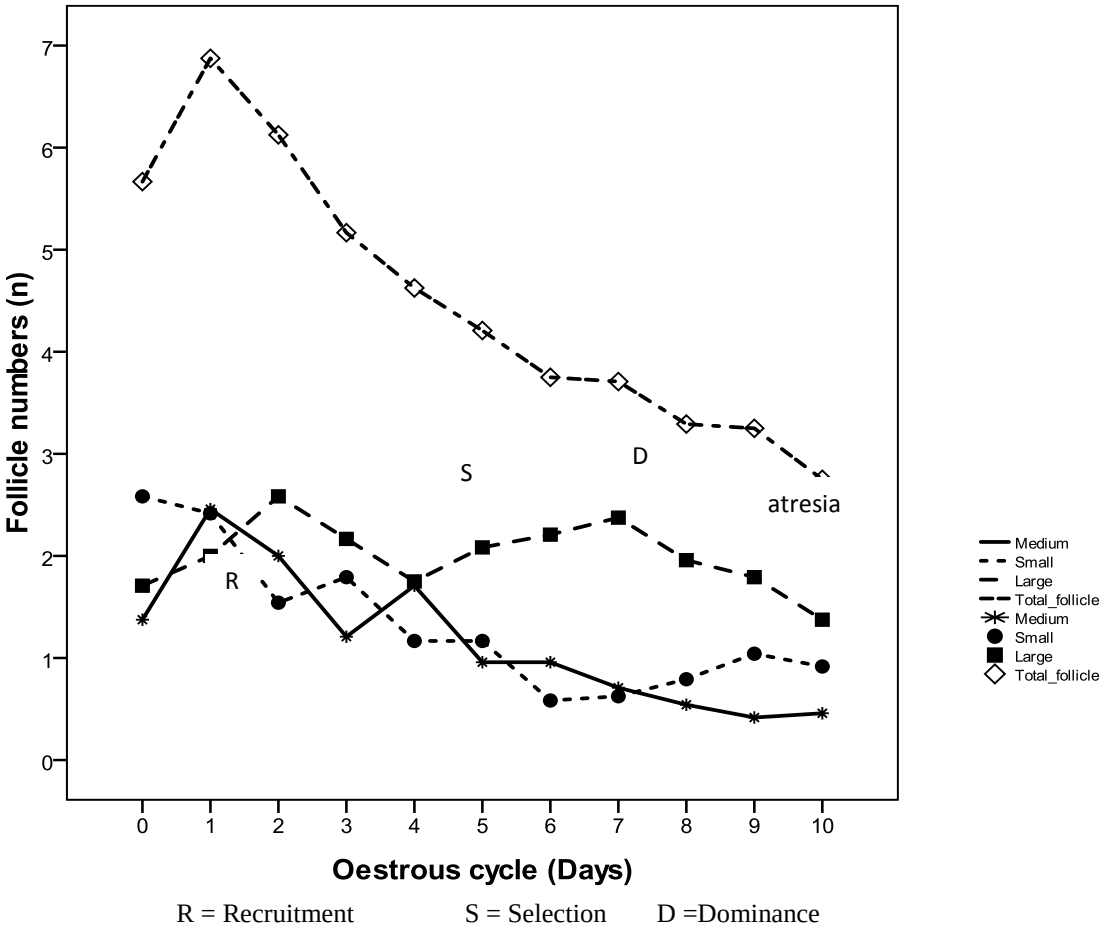
Tervit, H.R. 1996. Laparoscopy/laparotomy oocyte recovery and juvenile breeding. Anim. Reprod. Sci. 42: 227-238.

Wurth, Y.A., Boni, R., Hulshof, S.C.J. and Kruip, T.A.M. 1994. Bovine embryo-production after selection of ovaries, follicles and oocytes. In Wurth, Y.A. "Bovine embryo production in vitro. Influencing factors", Utrecht University Press, 67-85.



RegimeA: Treated intramuscularly with Follicle Stimulating Hormone (FSH) for 3 consecutive days beginning on day 4 of controlled internal drug releasing device (CIDR-B®) insertion;
RegimeB: Treated intramuscularly with FSH for 4 consecutive days beginning on day 4 of CIDR-B® insertion;
RegimeC: 2500 iu PMSGS' administered intramuscularly once on day 4 of CIDR-B® insertion;
RegimeD: Treated intramuscularly with FSH for 4 consecutive days beginning on day 9 of PGF injection.

Figure 1. Number of follicles obtained from daily follicular scanning during growth phase of first follicular wave development treated with various types of gonadotropin regimes.



R = Recruitment S = Selection D =Dominance

Figure 2. Mean number of follicles sizes during growth phase of first follicular wave development treated with various types of gonadotropin regimes.