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Endocrinological interventions and subsequent conception of repeat breeding cows having cystic ovarian follicles

JAGIR SINGH[1], D DADARWAL[2], M HONPARKHE[3], G S DHALIWAL[4] and V K GANDOTRA[5]

Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab 141 004 India

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ABSTRACT

The experiment was intended to evaluate the efficacy of various hormonal interventions for treating cystic ovarian follicles and subsequent conception in repeat breeding crossbred cows. Repeat breeding cows (22) with the history of frequent/constant estruses were subjected to rectal palpation of ovaries and blood samplings for plasma progesterone (P_4) profiles on day 0 (estrus), 5, 10, 15 and 20 of estrous cycle. The hormonal treatment was given to the cows on the basis of presence/absence of CL and P_4 profiles and subsequently divided into group 1 (n=5; control untreated), group 2 (n=8; GnRH alone treated) and group 3 (n=9; GnRH+ PGF2 α + GnRH treated). Group 1 cows were not treated and advised for breeding at next estrus. Group 2 animals had follicular cyst without CL (P_4 = <0.5 ng/ml up to day 5 of estrous cycle) and were administered with 20 µg buserelin acetate intramuscularly at the time of breeding during next estrus. Group 3 cows had follicular cyst along with CL on either of the ovaries (P_4= < 1 ng/ml up to day 10 of estrous cycle) were given 20 µg buserelin acetate intramuscularly at spontaneous estrus (day 0) followed by 25 µg dinoprost intramuscularly at 12 th day of estrous cycle followed by breeding at induced estrus along with administration of 20 µg buserelin acetate intramuscularly. The pregnancy diagnosis was done at 90 days post-breeding. Amongst control group (group 1), no cow was found pregnant. In group 2, 3/8 (37.5%) cows whereas, in group 3, 5/9 (55.5%) cows were pregnant. Hence it was concluded that GnRH+ PGF2 α + GnRH was more effective than GnRH alone to intervene repeat breeding due to cystic ovarian follicle.

Key words: Cystic ovary, Follicular cyst, Repeat breeding syndrome

Ovarian cysts are one of the most mystifying causes of repeat breeding in dairy cows leading to significant economic loss to dairy farmers in terms of increased calving to conception and calving intervals. A cystic follicle may persist (usually > 10 days) in the absence of any active luteal tissue clearly interfering with normal ovarian cyclicity (Vanholder *et al.* 2006). The incidence of ovarian follicular cyst in dairy cattle is reported to be 5.6–18.8% (Garverick 1997, Silvia *et al.* 2002). The mechanism/pathogenesis of cyst formation is not yet clear (Wiltbank *et al.* 2003, Vanholder *et al* 2005). It is believed that the cystic follicles develop due to a dysfunction of the hypothalamic-pituitary-ovarian axis. This dysfunction has a multifactorial etiology involving various clinical, environmental and genetic factors (Peter 1997, Zulu and Penny 1998, Peter 2000, Ribadu *et al.* 2000, Peter 2004). However, the most understood pathogenesis of ovarian cyst formation is the lack of pre-ovulatory LH surge or the failure of this surge to occur at right time during follicular maturation process (Vanholder *et al.* 2002, Peter 2004). In the presence of cystic follicles the occurrence of intermittent follicular waves were observed as a constant process (Wiltbank *et al.* 2003). The fate of the follicular cyst is either by losing dominance and new dominant follicles may develop and ovulate or they may be replaced by yet another cystic follicle (Garverick 1997). The prevailing progesterone profile could be the determining factor for cystic turnover (Silvia *et al.* 2002, Hatler *et al.* 2003). Conventionally, cystic ovarian follicles are diagnosed on the basis of reproductive history and the presence of thin walled, smooth, fluid filled structure $\geq$ 2.5 cm in diameter through rectal palpation. However, such diagnosis may not always be accurate because of variable characteristics (Youngquist 1986, Archbald and Thatcher 1992, Garverick 1997). The combination of ultrasonography, rectal palpation findings and determination of plasma progesterone profiles can improve the accuracy to diagnose cystic ovarian follicles (Douthwaite and Dobson 2000). To intervene the condition, various treatments have been investigated including GnRH, hCG, LH and P_4 interventions (Nanda *et al.* 1988, Savio *et al.* 1993, Stock and Fortune

Present address: [1]Senior Gynaecologist, [2,3]Assistant Gynaecologist, [4]Professor-cum-Head, Department of Animal Reproduction, Gynaecology and Obstetrics. [5]Professor, Department of Veterinary Clinical Service Complex.

1993, Diwakar *et al.* 2004, Peter 2004, Todoroki and Kaneko 2006) but the treatment outcomes are highly variable. Bartolome *et al.* (2000) indicated ovsynch (using two GnRH injections 9 days apart with $PGF_2\alpha$ after 7 days of first GnRH) followed by timed AI that improved conception rate but pregnancy rates were not encouraging . Therefore, the present study was aimed to evaluate the efficacy of exogenous hormonal supplementation including ovsynch for improving pregnancy rates in repeat breeding crossbred dairy cattle having cystic ovarian follicles.

MATERIALS AND METHODS

Selection of animals: Problem breeder crossbred (HF×Sahiwal) cattle (22) belonging to second –fourth parity were selected from different villages of Punjab on the basis of history of abnormal pattern of estrus behavior especially nymphomania/frequent estrus at short intervals. Cows were fed according to their requirements for maintenance and milk production. All the animals were subjected to rectal palpation on days 0 (day of estrus), 5, 10, 15 and 20 to confirm the persistence of follicular cyst as a thin walled fluctuating structure (>2.5 cm) in the presence or absence of CL on either of the ovaries. All the cows were confirmed for any other anatomical abnormalities of the genital tract. The cervico-vaginal mucus (CVM) was examined for presence of any type of turbidity. The cows with apparently clear CVM were selected. The body condition scores of the experimental cows were recorded by the same person on the day of start the study. Scoring was performed on a five-point scale (1=thin, 5=fat), with 0.25 increments as described by Edmondson *et al* 1989.

Blood sampling: Blood samples were collected on days 0 (day of estrus), 5, 10, 15 and 20 to analyse plasma concentration of progesterone (P_4) in the peripheral circulation. Samples were collected by jugular venipuncture into sterile, heparinized blood-collection tubes and transported to the laboratory immediately in an ice container. Blood plasma was harvested within 3 h of collection by centrifugation at 3000 rpm for 15 min and was stored at –20°C until assayed for progesterone at the laboratory.

Progesterone analysis: Plasma progesterone was analyzed by using precoated tube of RIA kits. The mean analyti sensitivity of the assay was 0.14ng/ml. The interassay a intraassay coefficients of variation for progesterone were and 7.3%, respectively.

Experimental groups: On the basis of presence/abse of CL, P_4 profiles and hormonal administration, the cc were divided into 3 groups viz. group 1 (n=5; cont untreated), group 2 (n=8; GnRH alone treated) and grou (n=9; GnRH+ $PGF_2\alpha$ + GnRH treated). Group 1 cows hav follicular cyst without (n=3; P_4 = <0.5 ng/ml up to day 1 estrous cycle) or with (n=2; P_4= <1.0 ng/ml up to day 1(estrous cycle) CL were not treated and advised for breed at next spontaneous estrus. Group 3 cows having follic cyst without CL (P_4 = <0.5 ng/ml up to day 15 of estr cycle) and were administered with 20 µg buserelin ace intramuscularly at the time of breeding during spontane estrus. Group 2 cows having follicular cyst along with (P_4= <1 ng/ml up to day 10 of estrous cycle) were given µg buserelin acetate intramuscularly at spontaneous es followed by 25 mg dinoprost intramuscularly at 12 th da estrous cycle followed by breeding at induced est along with administration of 20 µg Buserelin ace intramuscularly.

Pregnancy diagnosis: After inseminations, the cows w observed daily for signs of estrus, especially 18 to 25 d post AI. Eventual pregnancy status of those cows returning to estrus past this period was determined by re palpation at 90 days post-breeding.

RESULTS AND DISCUSSION

Progesterone profiles

In group 2, cows (without CL) the P_4 concentrat remained low up to day 15 of estrous cycle (Table Follicular cysts are generally associated with low periph P_4 concentration (Dobson *et al.* 1977, Farin *et al.* 1990, Si *et al.* 2002, Diwakar *et al.* 2004, Todoroki and Kaneko 20 The pulsatile LH secretion is higher in cystic cows bec of lack of negative feedback effects of P_4 than the le observed in normal luteal phase. The relatively high pulses are responsible for the excessive growth of foll (Stock and Fortune 1993). However, LH surge is lackin

Table 1. Plasma progesterone profiles (mean ± SE ng/ml) of repeat breeder cows with cystic ovarian follicles on different days of estrous cycle

Groups	Progesterone concentration (ng/ml) on different days of estrous cycle				
	0	5	10	15	20
Gp 1 (Control)					
without CL (n=3)	0.121±0.04	0.20±0.05	0.145±0.06	0.172±0.01	0.10±0
with CL (n=2)	0.28±0.09	1.68±0.29	1.95±0.5	1.00±0.7	0.22±0
GP 2 (n=8)					
Without CL	0.149±0.009	0.109±0,004	0.107±0.014	0.486±0.092	0.590±(
Gp 2 (n-9)					
With CL	0.335±0.07	0.489±0.10	0.815±0.68	1.968±0.64	0.698±(

cystic cows because of functional abnormalities in the estradiol feedback regulation of LH secretion. Therefore, there is prolonged growth of follicle but no ovulation occurs. Group 3 cows (with CL) had intermediate levels of P_4 up to day 10 of estrous cycle. These intermediate levels of P_4 caused hypothalamic insensitivity to estradiol resulting in to lack of LH surge thereby preventing ovulation in cystic cows (Silvia *et al* 2002). The possible sources of this progesterone are cyst itself (undergone partial luteinization), incompletely luteolysed CL of previous cycle and adrenal gland (Silvia *et al.* 2002, Diwakar *et al.* 2004, Vanholder *et al.* 2005).

Efficacy of GnRH alone (group 2)

Out of 8 cows in group 2, 3 were found pregnant rendereing first service pregnancy rate (FSPR) 37.5%. Of remaining 5 cows, 2 cows became pregnant (received GnRH again at the time of breeding at subsequent estrus) while 3 cows remained infertile during next estrus. The overall pregnancy rate was 31.2%. The GnRH treatment was given to induce the release of LH from anterior pituitary and ovulation. Very high success rate (50–80%) of GnRH treatment for inducing ovulation in cystic ovaries has been documented (Nanda *et al.* 1988, Dinsmore *et al.* 1989, Diwakar *et al.* 2004). The persistence of cystic follicles become non-steroidogenic and act as a benign structure after a variable period of time and then they no longer interfere with the cyclicity (Garverick 1999, Dobson *et al.* 2000, Noble *et al.* 2000), and it is highly unlikely to have any negative effect on new follicular development. However, poor response following same treatment in the present study suggestive of altered functions of pituitary-ovarian axis rendering it non-responsive to GnRH treatment (Jou *et al.* 1999, Peter 2004). It is also documented that some refractory follicular cysts do not respond to GnRH treatment (Woolums and Peter 1994, Peter 2004).

Efficacy of GnRH–PG- GnRH (group 3)

Following this treatment 5/9 (55.5%) cattle were found pregnant yielding 55.5% of FSPR. The remaining 4 cattle did not conceived during subsequent estrus. Following first GnRH treatment, a surge like release of LH occurs that results in luteinization of cystic follicles and/or ovulation of existing follicles in the presence of cystic follicles (Diwakar *et al.* 2004, Todoroki and Kaneko 2006) and thereafter administration of PGF_2 alpha causes lysis of luteinized structures (luteinized cysts or newly mature CL or previous cycle CL). Thus, the possible sources of high progesterone levels are slashed thereby and in response to the second GnRH treatment ovulation takes place (Bartolome *et al.* 2000, Peter 2004). In group 1 (control group) out of 5 cows none was found pregnant.

It was concluded that GnRH+ PGF2 α + GnRH was more effective than GnRH alone to intervene repeat breeding due to cystic ovarian follicle.

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