The Follicular Distribution during the Estrus Phase and the Enhanced Estrus Behavior of Crossed Ongole Heifers Stimulated with a Non-superovulation Dose of PMSG

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ABSTRAK

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Penelitian ini dilakukan untuk mempelajari sebaran folikel pada fase estrus dan kaitannya dengan performa estrus dan perubahan biometrik organ reproduksi sapi Peranakan Ongole (PO) dara yang diinjeksi *Pregnant Mare Serum Gonadotrophin* (PMSG) dengan dosis non-superovulasi (NSO). Sembilan ekor sapi PO dara dibagi ke dalam tiga kelompok secara acak berdasarkan dosis PMSG 0 (plasebo), 0.5, dan 1.0 IU/Kg BB yang diinjeksi pada awal gelombang folikel kedua. Dosis PMSG 0.5 dan 1.0 IU/Kg BB terbukti mampu meningkatkan rerata diameter folikel besar (> 0.8 cm) sebesar 7.08 % (P>0.05) and 15.04 % (P<0.05), akan tetapi total jumlah folikel besar pada dosis 1.0 IU/Kg BB masih tinggi (5.33±2.52, P<0.05) sehingga berpotesi menginduksi *multiple calving*. Skor performa estrus terbukti meningkat seiring dengan peningkatan dosis PMSG yang diikuti dengan perubahan biometrik organ reproduksi, khususnya ovarium, uterus, dan serviks (P<0.05). Hasil penelitian menunjukkan bahwa pemberian PMSG dengan dosis NSO mampu meningkatkan pertumbuhan dan perkembangan folikel bersamaan dengan peningkatan kualitas estrus tanpa meningkatkan risiko *multiple calving*.

Kata Kunci: Distribusi Folikel, Sapi PO Dara, Ovarium, PMSG, Biometrik Traktus Reproduksi

ABSTRACT

Putro KB, Amrozi, Winarto A, Boediono A, Manalu W. 2024. The follicular distribution during the estrus phase and the enhanced estrus behavior of crossed Ongole heifers stimulated with a non-superovulation dose of PMSG. JITV 29(4):172-180. DOI:http://dx.doi.org/10.14334/jitv.v29.i4.3160.

The experiment's objective was to study the follicle distribution during the estrus phase and the estrus performance of the Ongole-crossed heifer by injecting a non-superovulation (NSO) dose of PMSG. Nine PO heifers were randomly assigned to one of three treatment groups, each receiving a different dose of PMSG injection, i.e., 0, 0.5, and 1.0 IU/kg BW, respectively; this was done at the early second follicular phase. The evaluation was conducted during the estrus phase to assess follicle distribution and estrus performance and to collect the reproductive tract for biometrics evaluation. The results demonstrated that PMSG injection at doses of 0.5 and 1.0 IU resulted in an increase in mean follicle diameter size above 0.8 cm by 7.08 % (P>0.05) and 15.04 % (P<0.05), respectively. However, the total number of follicles above 0.8 cm at 1.0 IU/kg BW dose remains high (5.33±2.52, P<0.05), indicating a continued propensity for multiple calving. The results demonstrated that the estrus performance score was enhanced with the administration of increasing doses of PMSG, which was accompanied by an increase in the biometrics of the ovaries (P<0.05) and the diameter of the uterine body, uterine horn, and cervix (P<0.05). It was concluded that the NSO dose of PMSG could facilitate follicle development and enhance estrus quality without increasing the risk of multiple calving.

Key Words: Follicle Distribution, Ongole-crossed Heifers, Ovary, PMSG, Reproductive-tract Biometrics

INTRODUCTION

The Ongole-crossed cattle is an endemic breed distributed throughout Indonesia, particularly prevalent in the Central Java and East Java provinces. Ongole-crossed cattle represent the results of a grading-up breeding process between Java and Sumba Ongole (SO) cattle (Astuti 2004). Although the Ongole-crossed cattle results from a grading-up breeding program, this breed frequently encounters difficulties in terms of reproductive performance. Some reproductive performances categorized as low in this breed of cattle are anestrus, especially in heifers (Wahyunani 2014), calving rate, birth weight, weaning weight, and post-weaning growth rate of calves (Astuti 2004). These reproductive performances are markedly inferior to those observed in Bali cattle (Saili et al. 2011). The estrus cycle of heifers is often disrupted. Most heifers exhibit signs of anestrus during their initial and subsequent natural estrus cycles, with or without estrus synchronization. Ma'ruf et al. (2017) reported that more than 60% of Ongole-crossed heifers treated with medroxyprogesterone acetate (MPA) experienced anestrus. This problem frequently causes Ongole-crossed heifers to give birth at older ages, i.e., above 24 months (Astuti 2004).

The growth and development of follicles were stimulated by administering Pregnant Mare Serum Gonadotropin (PMSG) to enhance the livestock reproductive performance. Several studies have demonstrated that PMSG injection increases the endogenous secretion of estradiol in thin-tailed sheep (Sugiyatno et al. 2001) and has been shown to enhance the estrus performance of Boer goat (Salleh et al. 2021). In Chinese Holstein cows, estrus signs manifested in 70-80% of animals within 74-98 hours after administering a low dose of PMSG (Fu et al. 2013). Furthermore, gilts exhibited enhanced follicle development and estrus performance following PMSG injection during the luteal phase (Zeng et al. 2019), a phenomenon also observed in does (Kavitha et al. 2018).

The implementation of this methodology has the potential to enhance the reproductive and productive performance of mammalian animals markedly. Furthermore, offspring born to the mother stimulated by PMSG injection prior to mating exhibited elevated birth weights (Manalu et al. 1997; Manalu et al. 2000). The administration of PMSG to ewes prior to mating has been demonstrated to result in an average increase in weaning weights productivity in the small-scale farm (Manalu 2012). Furthermore, the efficacy of PMSG in augmenting endogenous secretion of pregnant hormones has been demonstrated in the sow (Rayer et al. 2015). This method also has been demonstrated to enhance lamb resilience to *H. contortus* (Arif et al. 2018).

The majority of experiments conducted on the enhancement of reproductive performance in Ongole-crossed cattle in Indonesia have been limited in their utilization of luteolytic agents and improved nutrition and have demonstrated a lack of comprehensive follicle distribution analysis during the estrus phase of monotocous ruminants. The administration of an NSO dose of PMSG at the second follicular wave in Ongole-crossed heifers represents a potential alternative method for enhancing the reproductive performance of Ongole-crossed cattle and mitigating the risk of multiple calving in monotocous animals. This experiment's objective was to study the follicle distribution during the estrus phase and the subsequent estrus performances, as well as biometric changes in the reproductive tracts of Ongole-crossed cattle in response to an NSO dose of PMSG.

MATERIALS AND METHODS

The experiment was conducted in the Laboratory Animal Management Unit, School of Veterinary Medicine and Biomedical Sciences, IPB University, and reproductive tracts were collected at the local slaughterhouse. The Animal Ethics Committee, School of Veterinary Medicine and Biomedical Science, IPB University, approved all experiments (approval number 090a/KEH/SKE/XI/2017).

Preparation of heifers and experimental design

Nine Ongole-crossed heifers, aged between two and a half years and with a body condition score of 2.5 to 3.0, were selected based on the aforementioned criteria. They were observed to have regular estrus cycles based on the observation using real-time ultrasonography (USG, Aloka model SSD 500, linear probe 7.5 MHz, Aloka Co. LTD, Tokyo, Japan). They were deemed to have satisfactory reproductive tract health. Over one month, the heifers were acclimatized to the experimental conditions by being fed a similar ration twice daily.

The experimental heifers were allocated to a completely randomized design comprising three treatments of dose of PMSG injection, namely 0.0, 0.5, and 1.0 IU/kg BW, each with three replications. The heifers were categorized into three groups, designated as D0.0, D0.5, and D1.0, respectively. Following the acclimatization period, heifers were subjected to an estrus synchronization procedure, whereby they were administered an injection of $PGF_{2\alpha}$ (Lutalyse, Zoetis, Dublin, Ireland) at a dosage of 25 mg/heifer in two separate intramuscular injections with an interval of 12 days between each injection. A PMSG injection (Folligon, MSD, Intervet BV, The Netherlands) was administered intramuscularly at the onset of the second follicular wave. The emergence of the second follicular wave was monitored via real-time ultrasound. The emergence of a cohort of small antral follicles (0.3-0.5 cm) was employed to indicate the advent of the follicular wave. Subsequently, the heifers were administered an injection of $PGF_{2\alpha}$ 48 hours later, which resulted in lysis of the existing corpus luteum (CL). This procedure ensured that the dynamics of the ovary continued uninterrupted until the estrus phase. The ultrasound examination continued until a dominant follicle and estrus mucus confirmed the appearance of estrus signs. All heifers that exhibited signs of estrus were humanely euthanized by slaughtering at a local abattoir, with the reproductive tract and the ovary collected for further analysis.

Follicle distribution

On the day that estrus was detected, an ultrasound scan (USG) was performed to evaluate the distribution of follicles in size across both ovaries. The number of follicles was counted and divided into three categories based on sizes: 0.20-0.59 cm (small), 0.60-0.80 cm (medium), and above 0.80 cm (large). Moreover, the mean diameter of the large follicles (above 0.80 cm) was determined to acquire information regarding ovarian dimensions and correlation with the propensity for an increased number of large follicle formations.

Observations of estrus's performances

The occurrence of estrus was determined through the observation and scoring of the estrus signs, employing the methodology described by Saili et al. (2011) and Abidin et al. (2012). The appearance of estrus was indicated by an increase in vaginal temperature, alterations in the vaginal coloration, and swelling of the vulva, which were classified as physical changes. Additionally, behavioral changes were observed, including mounting other cattle or remaining standing when mounted by the other cattle. The scoring was divided into three categories: score 1 was assigned when the physical change was incomplete, whether or not estrus mucus; score 2 was assigned when the physical change was complete, and estrus mucus was present; and score 3 was assigned when the physical change was complete, and estrus mucus was present, along with behavioral changes. Additionally, vaginal temperature was monitored as a further indicator, given its importance as a sign of estrus (Higaki et al. 2019). Vaginal temperatures of heifers were recorded by using a digital thermometer (GEA®) on the same day as the injection of PGF2α and monitored until the appearance of estrus.

Collection of the ovary and uterus

The reproductive tracts collected were of the following types: vulva-vagina, cervix, uterine body, uterine horn, and ovary. Macro-anatomical parameters were measured, including length, diameter, and thickness of the wall of the uterine horn, uterine body, and cervix. The ovary was measured for length, width, and thickness. The number of follicles on the surface of the ovary was counted.

The length of the uterine horn was determined by utilizing a stainless-steel pipe strap, measuring from the bifurcation's base to the uterine horn's extremity. Moreover, the length of the uterine horn was determined by measuring from the base of bifurcation up to the orifice of the cervix, which is in contact with the uterine horn, using a caliper. The length of the cervix was measured from the orifice of the cervix, which is the point of junction with the vagina, to the point of junction with the uterine horn. The whole diameter was also measured. The ovary was separated from the oviduct, and then the dimension of the ovary was measured.

Data analysis

The estrus performances and vaginal temperature were presented descriptively. The data about biometric observation of reproductive tracts and the ovary were subjected to the variance (ANOVA) test, with the results processed using the SPSS 16.0 software. The Duncan test was employed for further analysis when a statistically significant effect was observed.

RESULTS AND DISCUSSION

Follicle distribution

The experiment's results demonstrated a statistically significant difference in the distribution of follicles and the mean diameter of large follicles (above 0.8 cm), as illustrated in Table 1. The highest number of large follicles was found in D1.0 ($P<0.05$), while other groups showed the same results (2±0, $P>0.05$). These findings demonstrate that the highest PMSG dose in this experiment still had a propensity for multiple dominant follicle formation, thus increasing the risk of multiple calving. Moreover, the mean diameter of follicles exceeding 0.8 cm exhibited a notable elevation at the D1.0 dosage, whereas the D0.5 dosage did not reach statistical significance ($P>0.05$) despite an observed diameter increase up to 7.08%.

The experiment results demonstrated a correlation between the elevated NSO doses of PMSG administrations, the augmented prevalence of large follicles, and the dimensions of the ovaries. Pregnant Mare Serum Gonadotropin (PMSG) functions similarly to follicle-stimulating hormone (FSH), which promotes the growth of small follicles, and luteinizing hormone (LH), which induces ovulation in the dominant follicle, particularly in pre-ovulatory follicles (Fu et al. 2013; Depison et al. 2011). The injection of heifers with PMSG has been observed to increase the number of small follicles selected to become dominant, even at the lower dose (Fu et al. 2013). This increased the number and diameter of the pre-ovulatory dominant follicles. Moreover, the increase in the number and diameter of the pre-ovulatory dominant follicle resulted in an ovary enlargement during the estrus phase. Several studies have demonstrated that PMSG treatment in conjunction with estrus synchronization protocols has a considerable effect on the growth and size of the pre-ovulatory follicle

Table 1. Number of follicles based on the size at the estrus phase in experimental heifers injected with different doses of Pregnant Mare Serum Gonadotropin (PMSG)

Sizes of the follicles (cm)	Doses of PMSG injection (IU/kg BW)		
	0	0.5	1.0
0.20-0.59	35.00±15.58[a]	62.33±2.63[b]	34.33±6.65[a]
0.60 – 0.80	0±0[a]	0.33±0.47[a]	0.67±0.94[b]
Above 0.80	2±0[a]	2±0[a]	5.33±2.52[b]
The mean diameter of the follicle above 0.8 cm	1.13±0.20[a]	1.21±0.20[a]	1.30±0.48[b]

Different superscripts in the same row mean a significant difference ($P<0.05$)

in various mammals, including buffaloes (Jerome et al. 2016; Terzano et al. 2013), cows (Elmetwally 2021), and goats (Kavitha et al. 2018; Hameed et al. 2020). The results of the present experiment demonstrate that the presence of follicles, particularly large follicles, significantly influences the dimension of the ovary. The highest number of large follicles was observed in D1.0 heifers, significantly impacting the ovary's weight, length, width, and thickness.

Furthermore, the most notable alterations in ovarian dimensions, particularly in weight, were observed in the right ovary. This finding indicates that most follicle growth and development in the present experiment occurs in the right ovary. It has been demonstrated in several studies that the right ovary is more active than the left ovary in both heifers and cows (Pierson et al. 1987; Lopez-Gatius 1997). In heifers, the ovulation of the dominant follicle in the right ovary was observed to occur in 54.1% of cases during the first estrus and 59.6% during the second estrus. Heifers' ovulation in the right ovary is 56.5% of heifer cases (Geres et al. 2011). These findings were evaluated in the recent report through a retrospective study in calves, which demonstrated that the right ovary has a higher propensity for a more significant number of antral follicles, larger follicle diameter, greater intrafollicular fluid volume, and a larger follicle surface (Dangdubiyyan & Ginther 2019). The present study demonstrated that the dominant follicles are predominantly formed in the right ovary, as evidenced by a significantly higher weight in the heifers treated with 0.5 and 1.0 IU of PMSG/kg BW.

Estrus performances

The heifers in the control group exhibited the lowest score, indicating incomplete physical changes. The swelling of the vagina was not detected even though there was a change in vaginal color and an increase in vaginal temperature that was associated with the secretion of estrus mucus (Table 2). The D0.5 heifers showed a higher score compared to the control heifers. All the D1.0 heifers exhibited complete physical changes accompanied by behavioral alterations. The vaginal temperatures were comparable at the outset of the treatment period, with a mean of approximately 38.4°C. A notable increase was observed during the estrus phase, reaching a peak (Figure 1). The control heifers reached the peak vaginal temperature (38.67°C) on the third day after the $PGF_{2\alpha}$ injection or the fifth day following the administration of the PMSG injection. In contrast, the D0.5 and D1.0 heifers reached the peak vaginal temperatures on the sixth day after PMSG injection with an average of 38.73°C in the D0.5 heifers and 38.98°C in D1.0 heifers. The D0.5 and D1.0 heifers exhibited estrus signs 24 hours later than the control heifers.

The remaining outcomes of the present study corroborate the hypothesis that heifers administered a higher dose of PMSG exhibited more robust estrus-related behavior and has been demonstrated to have a positive correlation with plasma estradiol concentration (Lyimo 2000), which in turn increases with the stimulation provided by an increasing dose of PMSG. A previous study reported a significantly higher estrus response and duration following estrus synchronization with a combination of progesterone, prostaglandin, and PMSG (Kavitha et al. 2018). Tirpan et al. (2019) additionally observed that Angora goats exhibited heightened levels of estrus behavior following estrus synchronization with a PMSG combination protocol. These increasing estrus behaviors are highly correlated with the increasing diameter of the pre-ovulatory follicle diameter (Jitjumnong et al. 2019; Putro et al. 2020), followed by a higher level of plasma estradiol (Pandey et al. 2018; Mohamed et al. 2021; Hunter & Lopez-Gatius 2020). The aforementioned experiments demonstrate that when the plasma estradiol reaches its peak, estrus behavior manifests with considerable intensity. Therefore, stimulation of heifers with PMSG during the follicle wave 2 in this study will improve the observed estrus signs, thus increasing the probability of successful mating.

Furthermore, the vaginal temperatures observed in the present experiment demonstrated a pattern of increase and reached the peak temperature during the estrus phase. An elevated vaginal temperature has been demonstrated to be closely associated with hormonal regulation during the estrus phase in a range of species, including the Bligon goat (Widiyono et al. 2011), the

Table 2. Scoring of estrus performances of Ongole-crossed heifers injected with low doses of Pregnant Mare Serum Gonadotropin (PMSG)

Doses of PMSG injection IU/kg BW	Number of heifers	Estrus score*		
		1	2	3
0	3	2 (66.67%)	1 (33.33%)	-
0.5	3	-	2 (66.67%)	1 (33.33%)
1.0	3	-	-	3 (100%)

*Based on the number of heifers that showed the same score.

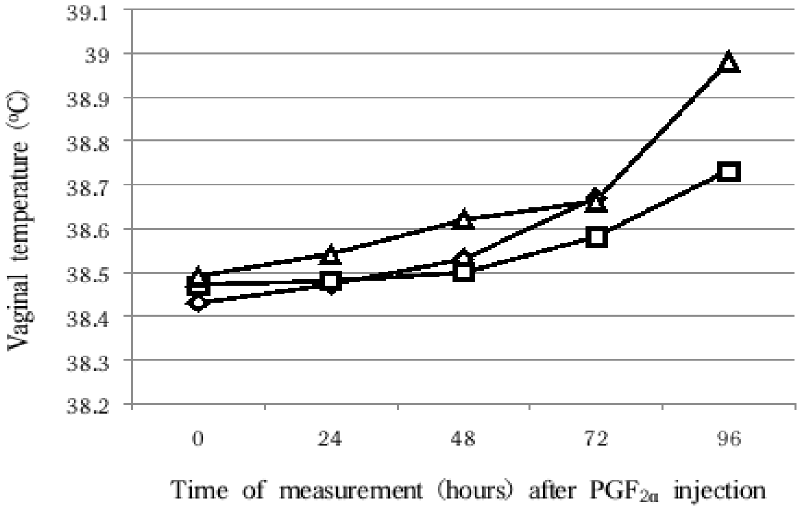

Figure 1. Vaginal temperatures of experimental Ongole-crossed heifers injected with PMSG at doses of 0 (—◇—), 0.5 (—□—), and 1.0 (—△—) IU/kg BW. Vaginal temperatures were measured from the injection of $PGF_{2\alpha}$ up to the appearance of estrus

dairy cow (Wang et al. 2020), camel (Mohamed et al. 2021), and other mammals (Lopez-Gatius 1997). The highest vaginal temperature was observed when the plasma estradiol reached its peak concentration, coinciding with the most complete and robust estrus signs exhibited by the heifers (score 3).

The other finding of the present experiment is the delayed onset of estrus signs following $PGF_{2\alpha}$ injection, with a mean of 4 days in D1.0 heifers versus 3 days in control heifers. The control heifers exhibited the typical time frame for the appearance of estrus signs following the administration of $PGF_{2\alpha}$ injection. It can, therefore, be concluded that the administration of PMSG has the effect of prolonging the duration of estrus appearance by approximately 24 hours. The delayed appearance of estrus in heifers injected with PMSG is likely attributable to the effect of PMSG on the corpus luteum regression. The mechanism of CL regression is postulated to have a relationship with a decreased level of nitric oxide (NO) and vascular endothelial growth factor (VEGF) (Ferrara et al. 1998; Jaroszewski & Hansel 2000; Shirasuna et al. 2012), as previously discussed in other research which indicates that VEGF plays an important role in CL formation by enhancing luteal angiogenesis (Chou & Chen 2018; Mara et al. 2020). Moreover, gonadotropin has been demonstrated to induce the expression of vascular endothelial growth factor (VEGF) in a dose- and time-dependent manner along with endothelin-2 (EDN2) mRNA, which is induced by luteinizing hormone (LH) (Shreshta et al. 2019). It is hypothesized that the injection of PMSG, acting as an agent of follicle stimulation, enhances the activity of VEGF, NO, EDN2, and other signaling agents, which in turn causes the late regression of the corpus luteum. A longer-lasting corpus luteum will impede the development of selected follicles into dominant follicles due to the elevated level of progesterone. This condition results in a delay in the formation of the dominant follicle, which in turn delays the attainment of peak estradiol concentrations is also delayed.

Biometrics of the ovary

Further observation of the ovary revealed significant differences in the weight and thickness of the ovary (Table 3). The weight and thickness of the ovary were found to increase in proportion to the doses of PMSG injection administered. The weight of the right ovary in heifers administered D0.5 and D1.0 was significantly higher than in those administered D0.0 (P<0.05). Nevertheless, no statistically significant difference was observed in the ovary weights between the D0.5 and D1.0 heifers. The weight pattern of the left ovary differed from that of the right ovary. The weights of the left ovary in D0.0 and D0.5 heifers were similar (P>0.05), and D0.5 and D1.0 heifers also had similar weights of the left ovary (P>0.05). However, the weights of the left ovary in D1.0 heifers were higher (P<0.05) than in D0.0 heifers.

No significant differences (P>0.05) were observed in the other parameters, namely the length and width of the ovary, among the doses of PMSG injection. The length of the right ovary in all groups of heifers showed values above 3 cm, while the average length of the left ovary was shorter than the length of the right ovary across all doses of PMSG injection. The width of the right and left ovaries had almost similar values, with the lowest value observed in the left ovary of control heifers and the highest value of the left ovary of D1.0 heifers. Although no significant difference was observed, the longer right ovary compared to the left ovary indicated a tendency for the right ovary to be more active and responsive to the stimulation of PMSG at a lower dose.

The highest mean values for ovarian thickness were observed in D1.0 heifers (P<0.05) (Table 3). However, in the right ovary, a significant difference in the thickness of the ovary between the D0.0 and D0.5 heifers and between the D0.5 and D1.0 heifers was not found (P>0.05). The D1.0 heifers exhibited a significantly greater ovarian thickness on the right side than the control. No significant difference was observed in the thickness of the left ovary between the D0.5 and D1.0 heifers (P>0.05). Nevertheless, the ovary's thickness was significantly higher in the D0.5 and D1.0 heifers than the control heifers (P<0.05).

The enlargement of the reproductive tract in heifers was associated with the increased doses of PMSG injection. The largest is observed in D1.0 heifers, followed by D0.5 and D0.0 heifers. In general, stimulation with PMSG has been observed to elicit effects in increased numbers and diameters of dominant follicles. An increase in the diameter of the dominant follicle has been observed to result in an elevation of plasma estradiol concentration during the estrus phase (Lopes et al., 2007). Plasma estradiol during estrus plays a direct role in inducing the activation of physiological secretion processes of the endometrium and intensifying protein-lipid metabolism (Bondranenko et al. 2019). This mechanism results in the secretion of estrus mucus, which affects the size of the reproductive tract (Laksi & Trilaksana 2020) and increases the probability of conception (Damarany 2020).

Biometrics of the reproductive tract

Most reproductive tract parameters exhibited no statistically significant differences (P>0.05) across the doses of PMSG injection, as illustrated in Table 4. No significant differences were observed in the length of the right uterine horn between D1.0 and D0.0 heifers or between D0.5 and D1.0 heifers (P>0.05). However, heifers administered the lowest dose of PMSG (D0.5) exhibited a greater length of the right uterine horn than those administered the highest dose (D1.0). The diameters of the left and right uterine horns, as well as the diameters of the uterine body and cervix, showed similar patterns, i.e., there was a significant increase in the diameter with the increased dose of PMSG injection (D0.5 and D1.0) compared to control heifers. However, the increased doses of PMSG injection from 0.5 to 1.0 IU/kg BW did not significantly increase the diameters of the right and left uterine horns, as well as the diameters of the uterine body and cervix (P>0.05). The diameter of

Table 3. Morphological characteristics of the experimental heifers injected with different doses of Pregnant Mare Serum Gonadotropin (PMSG)

Ovary	Parameters	Doses of PMSG injection (IU/kg BW)		
		0	0.5	1.0
Right	Weight (g)	6.57±0.80[a]	7.43±0.13[b]	7.78±0.44[b]
	Length (cm)	3.05±0.22[a]	3.22±0.06[a]	3.01±0.43[a]
	Width (cm)	2.12±0.05[a]	2.61±0.18[a]	2.43±0.37[a]
	Thickness (cm)	1.73±0.07[a]	1.98±0.16[ab]	2.44±0.20[b]
Left	Weight (g)	4.69±0.90[a]	6.69±0.18[ab]	7.01±0.08[b]
	Length (cm)	2.63±0.22[a]	2.82±0.89[a]	2.93±0.22[a]
	Width (cm)	2.03±0.22[a]	2.22±0.23[a]	2.64±0.40[a]
	Thickness (cm)	1.78±0.15[a]	2.14±0.04[b]	2.24±0.06[b]

Different superscripts in the same row mean significant difference (P<0.05)

Table 4. Morphological characteristics of the reproductive tracts of the experimental heifers injected with different doses of Pregnant Mare Serum Gonadotropin (PMSG)

Uterus		Parameters (cm)	Doses of PMSG injection (IU/kg BW)		
			0	0.5	1.0
Horn	Right	Length	14.93±0.32[b]	18.72±0.67[a]	17.35±2.30[ab]
		Diameter	1.43±0.22[a]	2.99±0.21[b]	3.58±0.16[b]
		Thickness	0.65±0.11[a]	0.64±0.03[a]	0.75±0.02[a]
	Left	Length	14.92±0.61[a]	17.06±0.39[a]	16.44±1.52[a]
		Diameter	1.98±0.21[a]	2.45±0.18[b]	3.49±0.15[b]
		Thickness	0.66±0.13[a]	0.68±0.05[a]	0.69±0.02[a]
Body		Length	7.22±1.67[a]	7.91±0.79[a]	6.99±0.83[a]
		Diameter	4.06±0.24[a]	5.58±0.71[b]	5.75±0.20[b]
		Thickness	0.74±0.07[a]	0.71±0.04[a]	0.79±0.02[a]
Cervix		Length	5.38±0.69[a]	6.16±0.33[a]	6.39±0.33[a]
		Diameter	4.14±0.45[a]	5.22±0.61[b]	5.82±0.08[b]
		Thickness	0.95±0.06[a]	1.09±0.15[a]	1.08±0.10[a]
Vagina		Length	18.56±2.66[a]	23.47±0.44[a]	22.37±0.64[a]
		Diameter	4.44±0.20[a]	4.59±0.22[a]	5.10±0.56[a]
		Thickness	0.83±0.07[a]	0.67±0.02[a]	0.83±0.22[a]

Different superscripts in the same row mean significant difference ($P<0.05$)

the uterine horn in the D0.0 heifers was below 2 cm. In contrast, in the D0.5 heifers, the diameter of the uterine horn ranged from 2 to 3 cm, and in the D1.0 heifers showed an average diameter above 3 cm.

The diameter of the uterine body was found to be significantly ($P<0.05$) smaller in control heifers compared to both D0.5 and D1.0 heifers, which exhibited the largest diameter of the uterine body. The D1.0 heifers had the highest diameter of the uterine body among the groups of doses of PMSG injection. The diameter of the cervix in heifers administered with PMSG demonstrated a positive correlation with the dosage of PMSG administered. The highest diameter of the cervix was shown by D1.0 heifers, which D0.5 heifers followed. The heifers in the D0.0 group exhibited the most minor diameter of the cervix among the group receiving PMSG injections.

The wall thickness of all heifer groups administered different doses of PMSG was similar ($P>0.05$). However, a significant increase ($P<0.05$) in diameter was observed with elevated doses of PMSG injection. Thus, the observed increase in diameter is a consequence of the increased volume of secreted estrus mucus. An increase in plasma estradiol concentrations during estrus can result in heightened tension of the endometrium, enhanced mucus secretion of the uterus, and alterations in the size, color, and temperature of the reproductive tract, particularly in the vulva and vagina (Forde et al. 2011). The observed phenomena indicate that a lower dose of PMSG can enhance the quality of estrus, ultimately increasing the probability of mating and conception success.

CONCLUSION

The results of the experiment and the subsequent analyses indicate that administration of lower doses of PMSG can enhance the quality and performance of estrus, manifested as an improvement in estrus behavior of the crossed Ongole heifers; this, in turn, facilitates the detection and confirmation of estrus through discernible changes in the dimensions of the reproductive tract. The present study's findings suggest that the observed phenomena enhance the success of mating and conception. This approach could be an alternative method to improve reproductive efficiency in monotocous animals, such as local buffalo and cattle.

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