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Influence of Controlled Breeding Techniques on Estrus Induction Response, Conception Rate and Plasma Progesterone Profile in Anoestrus Buffaloes

By B.B. Nakrani, M.T. Panchal, A.J. Dhami, K.K. Hadiya, J.A. Patel, R.K. Gosai & R.G. Shah
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Abstract- This investigation was aimed to evaluate the fertility response in 55 postpartum (>90 days) anoestrus rural buffaloes treated with three standard hormonal protocols (CIDR, Ovsynch and Crestar, n=15 each), keeping a group of untreated control (n=10), and the findings were compared with a group of normal cyclic control buffaloes (n=10). All the 15 (100 %) buffaloes in each group under CIDR, Ovsynch and Crestar protocols exhibited induced oestrus with prominent, moderate or weak oestrus signs within mean intervals of 65.00±1.55, 69.46±1.04 and 46.00±1.37 hrs, respectively, from PGF2**α** injection. The conception rates obtained at induced oestrus in buffaloes under CIDR, Ovsynch and Crestar protocols were 46.67, 53.33 and 33.33 per cent, respectively. The corresponding overall conception rates of three cycles were 66.67, 73.33 and 60.00 per cent.

Keywords: buffalo, anoestrus, treatment protocols, oestrus induction, conception rate, fertile oestrus induction interval.

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Influence of Controlled Breeding Techniques on Estrus Induction Response, Conception Rate and Plasma Progesterone Profile in Anoestrus Buffaloes

B.B. Nakrani α, M.T. Panchal σ, A.J. Dhami ρ, K.K. Hadiya ω, J.A. Patel ¥, R.K. Gosai § & R.G. Shah χ

Abstract- This investigation was aimed to evaluate the fertility response in 55 postpartum (>90 days) anoestrus rural buffaloes treated with three standard hormonal protocols (CIDR, Ovsynch and Crestar, n=15 each), keeping a group of untreated control (n=10), and the findings were compared with a group of normal cyclic control buffaloes (n=10). All the 15 (100 %) buffaloes in each group under CIDR, Ovsynch and Crestar protocols exhibited induced oestrus with prominent, moderate or weak oestrus signs within mean intervals of 65.00±1.55, 69.46±1.04 and 46.00±1.37 hrs, respectively, from $PGF_2\alpha$ injection. The conception rates obtained at induced oestrus in buffaloes under CIDR, Ovsynch and Crestar protocols were 46.67, 53.33 and 33.33 per cent, respectively. The corresponding overall conception rates of three cycles were 66.67, 73.33 and 60.00 per cent. These were achieved with the mean intervals from $PGF_2\alpha$ injection of 11.40 ± 4.65, 12.70 ± 5.13 and 10.88 ± 3.84 days among treated conceived buffaloes in three groups, respectively. In untreated anoestrus control (n=10), only 2 buffaloes exhibited spontaneous oestrus within 90 days of follow up and one conceived giving first service and overall pregnancy rates of 50.00 and 10.00 per cent, respectively. In normal cyclic control (n=10), the conception rates at first and overall of 3 cycles were 40.00 and 70.00 per cent, respectively, and the service period was of 105.67 ± 7.44 days among conceived ones. The overall mean plasma progesterone concentrations in true anoestrus buffaloes on day 0, 7, 9 (AI) of treatment and on day 21 post-AI were 1.13 ± 0.66, 4.97 ± 1.68, 0.73 ± 0.67 and 3.47 ± 1.89 ng/ml, respectively, in CIDR protocol; 1.09 ± 0.15, 3.75 ± 0.47, 0.58 ± 0.10 and 4.06 ± 0.47 ng/ml in Ovsynch and 1.12 ± 0.36, 1.28 ± 0.15, 0.66 ± 0.10 and 2.44 ± 0.44 ng/ml in Crestar protocol, respectively. The values on day 7 and 21 were significantly (P<0.01) higher than other two periods in all three groups. The mean plasma progesterone concentrations were non-significantly (P>0.05) higher in conceived than non-conceived group on day 21 post-AI in CIDR (4.41 ± 1.62 vs. 2.66 ± 1.80 ng/ml), Ovsynch (4.14 ± 0.57 and 2.96 ± 0.82 ng/ml) and Crestar (3.21 ± 0.30 vs. 2.05 ± 0.61 ng/ml), but no differences were observed on day 0, 7 or 9 of treatment in any group. The high plasma progesterone in non-pregnant animals on day 21 post-AI was attributed to early embryonic mortality and irregular short or long cycle in them. However in normal Cyclic Control group, the mean plasma P_4 value on day 21 post-AI was significantly (P<0.05) higher in conceived buffaloes than in non-conceived ones (3.86 ± 0.47 vs. 1.18 ± 0.52 ng/ml). Thus, Ovsynch and/or CIDR protocols can be conveniently used to improve fertility in anoestrus rural buffaloes by the practicing veterinarians with results similar or even better than in normal cyclic buffaloes.

Keywords: buffalo, anoestrus, treatment protocols, oestrus induction, conception rate, fertile oestrus induction interval.

I. Introduction

The postpartum anoestrus is the most prevalent reproductive problem in dairy animals, for which several hormonal preparations and protocols are being practised by the field veterinarian, but with inconsistent results. Hormonal therapies have good therapeutic value to enhance reproductive efficacy in infertile animals with good nutritional status (Ghuman *et al.,* 2009; Malik *et al.,* 2010, 2011; Chaudhari *et al.,* 2012; Bhoraniya *et al.,* 2012; Parmar, 2013; Savalia *et al.,* 2014). The variable results obtained following hormonal treatments by different workers may be largely due to nutritional status, faulty management, ovarian changes, endocrine events and even uterine infection. Use of hormonal protocols like Ovsynch, CIDR and Crestar during breeding season can be helpful in inducing and synchronizing oestrus and getting better conception rate in them with lesser number of services per conception and making acyclic buffaloes to cycle normally, thereby achieving ideal inter-calving interval. Plasma progesterone levels denote either the presence or absence of CL and its functional competency which is directly related with fertility of the female. The progesterone hormone is responsible for stimulation of cyclicity, follicular development and also for continuation of pregnancy. Hence, this study was planned under field conditions to evaluate the comparative efficacy of CIDR, Ovsynch and Crestar protocols in anoestrus buffaloes for fertility enhancement and their influence on plasma progesterone profile.

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II. Materials and Methods

This study was carried out during breeding season from November, 2013 to March, 2014 on 55 postpartum (>90 days) anoestrus buffaloes and 10 normal cyclic buffaloes of average BCS selected from tribal areas of Gujarat. The buffaloes were initially screened gynaeco-clinically for their reproductive status as cyclic, anoestrus and detailed history and rectal palpation findings were recorded. Anoestrus buffaloes were confirmed by rectal palpation of small smooth inactive ovaries twice 10 days apart. All the selected buffaloes were initially dewormed using Ivermectin, 100 mg s/c. Owners of the ear-marked animals were supplied with multi-mineral boluses (Bolus-Minotas, Intas Pharma) for oral supplementing to their animals @ one bolus daily for 7 days. The anoestrus buffaloes were then randomly subjected to different standard estrus induction/synchronization protocols (viz., CIDR, Ovsynch and Crestar, n=15 each) with fix timed AI (Ghuman *et al.,* 2009; Naikoo *et al.,* 2010; Savalia *et al.,* 2014). Another 10 anoestrus animals were kept as anoestrus control and 10 normal cyclic buffaloes served as normal cyclic control group. Buffaloes in spontaneous or induced oestrus were inseminated using good quality frozen-thawed semen. Buffaloes detected in oestrus subsequent to FTAI were re-inseminated up to 3 cycles and in nor-return cases pregnancy was confirmed per rectum 60 days of last AI. All the hormonally treated/untreated true anoestrus and normal cyclic buffaloes were studied for their reproductive status and plasma progesterone profile. For this, jugular blood samples were collected in heparinized vacutainers four times from true anoestrus animals, i.e., on day 0 - just before treatment (on diagnosis), on day 7 - at the time of $PGF_2\alpha$ administration, on day 9 - induced oestrus/FTAI (FTAI done twice 24 hrs apart, i.e. on day 9 and 10 after initiation of treatment) and on day 21 post-AI. Blood sampling for two control groups of animals was done on the day of spontaneous oestrus if any, and on day 21 post-AI. The samples were centrifuged at 3000 rpm for 15 min. and plasma separated out was stored deep frozen at -20°C with a drop of merthiolate (0.1%) until analyzed. Plasma progesterone profile was estimated by using standard Radio-Immuno-Assay (RIA) technique of Kubasic *et al.* (1984). Labelled antigen (I^{125}), antibody coated tubes and standards were procured from Immunotech, France. The sensitivity of assay was 0.1ng/ml. The intra- and inter-assay coefficients of variation were 5.4 and 9.1 per cent, respectively.

The data on oestrus response, conception rate (by Chi square test) and plasma profile of progesterone (ANOVA) were analyzed statistically (Snedecor and Cochran, 1994) using online SAS software version 20.00.

III. Results and Discussion

a) Estrus Induction and Conception Rates

The oestrus induction response and conception rates at induced oestrus and overall of 3 cycles in animals under different hormonal treatment protocols are presented in Table 1. The cent per cent buffaloes in each group under CIDR, Ovsynch and Crestar protocols exhibited induced oestrus with prominent, moderate or weak oestrus signs within mean intervals of 65.00±1.55, 69.46±1.04 and 46.00±1.37 hrs, respectively, from the time of $PGF_2\alpha$ injection. The occurrence of prominent oestrus signs was observed in 66.67, 60.00, and 73.33 per cent of buffaloes in three groups, respectively, and it was statistically similar to the normal cyclic control group. The conception rates obtained at induced oestrus in buffaloes under CIDR, Ovsynch and Crestar protocols were 46.67, 53.33 and 33.33 per cent, respectively. The corresponding CRs at second cycle were 25.00, 28.57 and 30.00 per cent and at third cycle, 16.67, 20.00 and 14.28 per cent. The overall conception (pregnancy) rates of all three cycles were observed to be 66.67, 73.33 and 60.00 per cent, respectively in CIDR, Ovsynch and Crestar protocols. These pregnancy rates were achieved with the mean time intervals from $PGF_2\alpha$ injection of 11.40±4.65, 12.70±5.13 and 10.88±3.84 days among treated conceived buffaloes in three groups, respectively. In untreated Anoestrus Control group (n=10), only 2 buffaloes exhibited spontaneous oestrus within 90 days of follow up and one conceived at first AI (CR, 50.00 %) at 157 days postpartum giving overall pregnancy rate of only 10.00 (1/10) per cent. In normal Cyclic Control group (n=10), the conception rates at first, second, third cycle and overall of 3 cycles were 40.00, 33.33, 25.00 and 70.00 per cent, respectively, and the service period was of 105.67±7.44 days among conceived ones.

The mean oestrus induction intervals observed in buffaloes under CIDR and Ovsynch protocols under study compared favourably with the previous reports of Savalia *et al.* (2014) as 63.60 ± 6.46 and 70.67±6.15 hrs and Dhami *et al.* (2014) as 66.00±4.23 and 87.27±3.53 hrs in anoestrus buffaloes, and Patel *et al.* (2013) as 66.00±3.22 and 86.67±3.33 hrs in anoestrous crossbred cows, respectively, using the same protocols. Kundalkar *et al.* (2014) however, reported these intervals to be much shorter as 44.00±2.92 and 44.99±2.50 hrs, while Azawi *et al.* (2012) reported comparatively longer oestrus induction intervals with Ovsynch as 122.8±6.3 hrs. The mean oestrus induction interval found in buffaloes under Crestar ear implant protocol compared favourably with the previous reports of Utage *et al.* (2010) as 42.38±11.09 hrs and Dodamani *et al.* (2011) as 2.47±0.73 days, but Nath *et al.* (2004) observed it as 30.81±1.43 hrs only in anoestrus animals.

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The first service conception rates of the present study are comparable with the earlier results of Bhoraniya *et al.* (2012) as 66.66 and 33.33 per cent in CIDR and Ovsynch protocols in anoestrus Kankrej cows, and Kundalkar *et al.* (2014) as 42.50 and 50.00 per cent with same two protocols in buffaloes, although comparatively lower first service conception rates of 40.00 and 30.00 per cent (Savalia *et al.,* 2014), and 36.84 and 29.41 per cent (Dhami *et al.,* 2014), respectively, are documented with CIDR and Ovsynch protocols in recent reports also. Further, Malik *et al.* (2010. 2011) recorded overall conception rates of 85.70, 75.00 and 86.67 per cent in CIDR, Ovsynch and Crestar group, respectively, in anoestrus buffaloes, which are very close to the present finding with Ovsynch protocol (73.33 %), but far higher than in Crestar group (60.00 %). Ozyurtlu *et al.* (2009) reported overall conception rates of 44.00 and 53.85 per cent in Norgestomet and PRID groups, respectively, which are relatively lower than the present findings with Crestar and CIDR.

The overall conception rates in Ovsynch and CIDR protocol documented by Barucelli *et al.* (2003) as 52.50 and 28.20 per cent, Naikoo *et al.* (2010) as 50.00 and 50.00 per cent and Ali *et al.* (2012) as 60.00 and 33.33 per cent, respectively, are also lower than the present findings. Further, around 30 per cent conception obtained at second cycle in anoestrus buffaloes induced to cycle and even in normal cycling buffaloes, proved that all the oestrus induction and synchronization protocols induced oestrus and then established normal cyclicity in treated animals, resulting into conceptions in subsequent cycles like normal cycling/breeding buffaloes. These observations further supported the previous observations on use of similar protocols in anoestrus cows and buffaloes by many workers (Naikoo *et al.*, 2010; Bhoraniya *et al.*, 2012; Ammu *et al.,* 2012[a]; Chaudhari *et al.* 2012; Patel *et al.,* 2013; Savalia *et al.*, 2013, 2014; Dhami *et al.,* 2014).

The true anoestrus buffaloes thus could be induced to estrus within 2-3 days from the day of PG injection in each protocol and made pregnant within a period of 10-12 days in comparison to 125 days recorded in untreated control group, indicating a huge curtailment in the waiting period of 113 days for anoestrus animals to evince estrus and become pregnant, by putting then under such oestrus induction and synchronization protocols. The pooled conception rates obtained (66.67%) in the anoestrus buffaloes, irrespective of protocols used, indicated the positive contributory role of handling the problem of acyclicity in buffaloes for their induction of oestrus and making them pregnant to the levels, which is nearly at par with normal cyclic control buffaloes (70.00%). Based on the comparative conception rates obtained at induced/first oestrus, it can be surmised that Ovsynch and CIDR protocols could induce equally good fertile oestrus in anoestrus buffaloes. On the other hand, the frequency of induced fertile estrus was considerably low in Crestar protocol. The similar trend was also seen in overall pooled conception rates among the three protocols tested (Table 1).

Table 1 : Effects of CIDR, Ovsynch and Crestar protocols on oestrus induction response, PG injection to induced oestrus and fertile oestrus intervals, and conception rates in anoestrus buffaloes

Treatment Groups	No.	Oestrus Induction Response (%)	PG Inj. to Oestrus Interval (hrs)	Conception Rate (%)				PG Inj. to Fertile Oestrus Interval (days) among conceived ones
				Induced/ First Oestrus	Second Cycle	Third Cycle	Overall of 3 Cycles	
CIDR Protocol	15	100.00 (n=15)	65.00±1.55 (n=15)	46.67 (7/15)	25.00 (2/8)	16.67 (1/6)	66.67 (10/15)	11.40±4.65 (n=10)
Ovsynch Protocol	15	100.00 (n=15)	69.46±1.04 (n=15)	53.33 (8/15)	28.57 (2/7)	20.00 (1/5)	73.33 (11/15)	12.70±5.13 (n=11)
Crestar Protocol	15	100.00 (n=15)	46.00±1.37 (n=15)	33.33 (5/15)	30.00 (3/10)	14.28 (1/7)	60.00 (9/15)	10.88±3.84 (n=9)
Pooled	45	100.00 (n=45)	60.15±7.19 (n=45)	44.44 (20/45)	28.00 (7/25)	16.67 (3/18)	66.67 (30/45)	11.66±0.54 (n=30)
Untreated Anoestrus Control	10	20.00 (n=2)	--	50.00 (1/2)	--	--	10.00 (1/10)	157.00* (n=1)
Normal Cyclic Control	10	100.00 (n=10)	--	40.00 (4/10)	33.33 (2/6)	25.00 (1/4)	70.00 (7/10)	105.67±7.44*

*Figures in parenthesis indicate number of animals/observations, * Service period/days open*

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Thus, the buffaloes waiting for spontaneous cyclicity beyond 100 days postpartum can be the most appropriate candidates to be subjected to any of the above oestrus induction and synchronization protocols, and CIDR or Ovsynch in particular, for saving their valuable days of reproductive life span at field level, and making them early pregnant and productive.

b) Plasma Progesterone Profile

The mean levels of plasma progesterone recorded on day 0, 7, 9 (AI) of treatment and on day 21 post-AI in buffaloes under CIDR, Ovsynch and Crestar protocols, and on day of AI and day 21 post-AI in control groups are presented in Table 2. The data show that the mean plasma progesterone (ng/ml) concentrations were low towards basal values on day 0, i.e., on the day of initiation of treatment in all three groups, suggesting that the animals were in anoestrus phase. These levels subsequently rose significantly ($P<0.01$) to the peak values on day 7 (4.97±1.68, 3.75±0.47 and 1.28±0.15 ng/ml), particularly in animals under CIDR and Ovsynch protocols. i.e. just before implants were removed and PG was injected. Thereafter the mean progesterone levels dropped suddenly and significantly within 48 hrs of PG injection and/or implant removal to the basal values coincident to induced oestrus, when FTAIs were done. These levels again increased significantly ($P<0.01$) on day 21 post-AI in all the groups (3.47±1.89, 4.06±0.47 and 2.44±0.44 ng/ml) due to oestruses being ovulatory with development and maintenance of CL and establishment of pregnancy in some animals. In normal cyclic control group also the mean plasma progesterone concentration was the lowest (0.43±0.13 ng/ml) on the day of spontaneous oestrus/AI, which rose significantly ($P<0.05$) on day 21 post-AI (2.26±0.56 ng/ml) again due to establishment of pregnancy in four buffaloes in that cycle.

The mean plasma progesterone levels obtained on the day of initiation of CIDR and Ovsynch treatments in the present study corroborated with the earlier findings of Savalia *et al.* (2014) to be 0.55 ± 0.21 and 0.56 ± 0.23 ng/ml, respectively, in anoestrus buffaloes, however the levels varied from other reports of Ammu *et al.* (2012[b]) to be 0.81 ± 0.38 and 2.92 ± 1.19 ng/ml in Gir cows, and Patel *et al.* (2013) to be 0.65± 0.23 and 0.28±0.06 ng/ml in crossbred cows, with the same protocol. Significant rise observed in plasma P_4 profile on the day 7 of treatments in the present study with CIDR, Ovsynch protocols (4.97±1.68 and 3.75±0.47 ng/ml) over initial (0 day) values, with sudden drop to almost basal values on induced oestrus within 48-60 hrs after PG injection, has also been reported in anoestrus buffaloes by Patel *et al.* (2013)) and Savalia *et al.* (2013) and in cows by Ammu *et al.* (2012[b]) and Bhoraniya *et al.* (2012) by employing CIDR and Ovsynch protocol. It was, however, difficult to find any comparable report on progesterone profile following use of Crestar implant to support or defit the present observations. The apparently higher mean levels of progesterone found on day 21 post-AI in non-conceived buffaloes covered under CIDR, Ovsynch and Crestar protocols (2.66±1.80, 2.96±0.82 and 2.05±0.61 ng/ml, respectively) are suggestive of possibility of either prolonged cycles due to extended luteal phase/delayed luteal regression and/or delayed embryonic death.

Significantly higher mean plasma progesterone level (4.97±1.62 ng/ml) recorded on day 7 in CIDR group might be due to the continuous release of the exogenous progesterone from the progesterone molded silastic coil inserted in the anterior vagina of the buffaloes. In the Ovsynch protocol the rise in mean progesterone level (3.75±0.47 ng/ml) noted on day 7 might be due to luteinization of some of the growing follicles and/or ovulation of dominant follicle and formation of CL under the influence of GnRH, simulating diestrum phase, while in the Crestar protocol the mean progesterone level (1.28±0.15 ng/ml) did not rise much, probably due to presence of synthetic progestagen in that which is not detected by 17α-hydroxyprogesterone RIA, and the behavioural oestrus signs observed might also be attributed to i/m injection of oestradiol valerate simultaneous to norgestomet implant.

Further, the mean plasma progesterone concentrations in conceived and non-conceived groups in all three treatment protocols and in normal cyclic control group were found to be statistically similar on day 0, 7 and even on day 9 (AI), but on day 21 post-AI, the conceived buffaloes had non-significantly higher mean plasma progesterone concentrations as compared to non-conceived ones in all the three groups, but differed significantly only in normal cyclic Control group (3.86±0.47 vs 1.18±0.52 ng/ml, $P<0.05$) (Table 2). These findings on plasma progesterone profile with respect to effect of CIDR and Ovsynch protocols and/or in normal cyclic group closely corroborated with the observations of Raghorte *et al.* (2009), Naikoo *et al.* (2010) and Savalia *et al.* (2013, 2014) in anoestrus buffaloes, and of Bhoraniya *et al.* (2012) and Patel *et al.* (2013) in anoestrus cows under such protocols.

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Table 2 : Plasma progesterone concentrations (ng/ml) in anoestrus and cyclic buffaloes on different days of treatment/AI under various oestrus induction protocols

Treatment Groups	Pregnancy Status	No.	Days from initiation of treatment/AI			
			D-0	D-7	D-9 (AI)	D-21 post-AI
CIDR	Conceived	7	1.10±0.51	4.87±1.58	0.53±0.33	4.41±1.62
	Non-conceived	8	1.16±0.82	5.05±1.87	0.89±0.86	2.66±1.80
	Overall	**15**	**1.13±0.66^{a}**	**4.97±1.68^{b}**	**0.73±0.67^{a}**	**3.47±1.89^{b}**
Ovsynch	Conceived	8	0.94±0.18	4.01±0.74	0.47±0.11	4.14±0.57
	Non-conceived	7	1.26±0.25	3.46±0.58	0.70±0.17	2.96±0.82
	Overall	**15**	**1.09±0.15^{a}**	**3.75±0.47^{b}**	**0.58±0.10^{a}**	**4.06±0.47^{b}**
Crestar	Conceived	5	1.77±1.08	0.88±0.18	0.56±0.07	3.21±0.3
	Non-conceived	10	0.81±0.09	1.49±0.18	0.70±0.16	2.05±0.61
	Overall	15	**1.12±0.36^{a}**	**1.28±0.15^{b}**	**0.66±0.10^{a}**	**2.44±0.44^{b}**
Untreated Anoestrus Control	Conceived	1	0.63±0.00	--	0.34±0.00	4.17±0.00
	Non-conceived	9	1.37±0.49	--	--	--
	Overall	10	**1.07±0.41**	--	--	--
Normal Cyclic Control	Conceived	4	--	--	0.16±0.03	3.86±0.47^{x}
	Non-conceived	6	--	--	0.61±0.18	1.18±0.52^{y}
	Overall	10	--	--	**0.43±0.13^{a}**	**2.26±0.56^{b}**

Means bearing uncommon superscripts within the row / column differ significantly ($P < 0.05$).
D-0 = Day of starting the treatment, D-7 = Administration of PG, D-9= Fixed time AI,
D-21 = Day 21 post-AI

The levels of plasma P_4 on the day of beginning of treatment protocol helped delineate the reproductive and endocrine status of the animals and thereby predicting the possible response to the therapy. The higher plasma P_4 recorded on day 21 post-AI in conceived buffaloes of all the treatment groups and even in normal cyclic control group was due to establishment of pregnancy and maintenance of CL function, while significantly low yet variable plasma P_4 noted on day 21 post-AI in non-conceived buffaloes could be due to their return to next oestrus at varying intervals on account of probable irregular or long cycle length, early embryonic mortality after day 17 or uncoordinated, unexplained hormonal changes in some of them.

Thus, it can be inferred that the hormonal protocols used, particularly Ovsynch & CIDR protocol, improved conception rates in anoestrus buffaloes under field condition, and also influenced the plasma progesterone profile significantly in a manner of normal cyclic animals, hence can be used by the practicing veterinarians in anoestrus field buffaloes to improve their reproductive efficiency and thereby the farmers economy.

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Multi Drug Resistant Pseudomonas Aeruginosa: A Secondary Invader and Cause of Mortality in Foot-and-Mouth Disease Outbreak

By Amit Kumar Verma, Amit Kumar, Neha, Anu Rahal & Basanti Bist

Abstract- Foot-and-mouth disease is a highly contagious viral disease of the cloven-hoofed animals leading to severe economic losses to livestock industry. The disease is clinically characterized by pyrexia, vesicles on the mouth, muzzle, tongue, teats, inter digital space etc with high morbidity and low mortality in affected adults. However, the immune-suppression due to Foot-and-mouth disease virus may lead to development of secondary bacterial infection in the affected animals as a cause of mortality. Many of such secondary bacterial invaders have been reported. The present study revealed Pseudomonas spp. as monoculture from an outbreak leading to mortality in cattle and buffaloes. Pseudomonas aeruginosa, a ubiquitous bacterium, known to cause nosocomial infections such as pneumonia, urinary tract infections, and respiratory system infections are supposed to produce high case fatality rate in immune-suppressed host due to severe toxaemia and drug resistance. The isolates were subjected to antibiotic sensitivity test.

Keywords: animal, FMDV, pseudomonas, treatment, control.

GJMR-G Classification : NLMC Code: QW 45

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Multi Drug Resistant *Pseudomonas Aeruginosa:* A Secondary Invader and Cause of Mortality in Foot-and-Mouth Disease Outbreak

Amit Kumar Verma [α], Amit Kumar [σ], Neha [ρ], Anu Rahal [ω] & Basanti Bist [¥]

Abstract- Foot-and-mouth disease is a highly contagious viral disease of the cloven-hoofed animals leading to severe economic losses to livestock industry. The disease is clinically characterized by pyrexia, vesicles on the mouth, muzzle, tongue, teats, inter digital space etc with high morbidity and low mortality in affected adults. However, the immune-suppression due to Foot-and-mouth disease virus may lead to development of secondary bacterial infection in the affected animals as a cause of mortality. Many of such secondary bacterial invaders have been reported. The present study revealed *Pseudomonas* spp. as monoculture from an outbreak leading to mortality in cattle and buffaloes. *Pseudomonas aeruginosa*, a ubiquitous bacterium, known to cause nosocomial infections such as pneumonia, urinary tract infections, and respiratory system infections are supposed to produce high case fatality rate in immune-suppressed host due to severe toxaemia and drug resistance. The isolates were subjected to antibiotic sensitivity test. Of the 10 antibiotics tested, bacteria were highly resistant to amoxyclav, enrofloxacin, ofloxacin, levofloxacin, intermediate sensitive to penicillins, gentamicin and tylosine, and sensitive to amikacin, ceftriaxone+tazobactum and cefotaxim. The present study concludes that FMD outbreak was followed by secondary bacterial infections of *Pseudomonas aeruginosa*, which might have entered in the circulation through the lesions in tongue and foot. Moreover, immunosuppression due to FMD further led to colonization of *Pseudomonas aeruginosa* in critical body organs, such as the lungs, heart and kidney leading to severe mortality. Hence, the control of secondary invaders should be considered on priority to avoid the mortality in the outbreak situations.

Keywords: animal, FMDV, pseudomonas, treatment, control.

I. Introduction

Foot-and-mouth disease (FMD) is a highly contagious and devastating viral disease of the cloven-hoofed animals including cattle and buffaloes; and considered as a serious threat to the economy of the livestock industry all over the world the world (Verma *et al.,* 2008; Rodriguez and Gay, 2011; Verma *et al.,* 2012; Ding *et al.,* 2013; Xu *et al.,* 2013; Chakraborty et al., 2014). The disease is clinically characterized by pyrexia, vesicles on the mouth, muzzle, tongue, teats, inter digital space of feet and other hairless parts of skin (Teifke *et al.,* 2012). The morbidity rate is high almost reaching to 100% but mortality rate is low approximately 10% in adult animals but may reach upto 50% in young animals (claves) due to myocarditis (Gulbahar *et al.,* 2007; Raies *et al.,* 2009; Verma *et al.,* 2010a,b, 2012; Chakraborty *et al.,* 2014). The immune-suppression due to FMDV usually leads to development of secondary bacterial infection in the affected ignored animals. The bacteria of genus *Pseudomonas* are ubiquitous in nature and are well known to cause nosocomial infections. Drug resistance and severe toxaemia due to *Pseudomonas* infections are supposed to produce high case fatality rate in immune-suppressed host (Blanc *et al.,* 1998; Geyik, *et al.,* 2003; Hamud-Socoro, 2004). There are many factors which are responsible for *P. aeruginosa* infection in cattle and buffaloes, particularly exposure of open wounds with contaminated soils and water. Under stress, weak and debilitated animals suffering from skin injuries may lead to adherence of *Pseudomonas* with the skin surface and may contaminate the wound and form abscess. Drug resistance of the organism supports bacterial survival in wounds and further entry in circulation leading to involvement of multiple system viz., respiratory and urinary tracts. In advanced stages of the infection severe toxaemia leads to mortality. Hence, the aim of the present study was to reveal the cause of mortality in cattle and buffaloes following to an outbreak of FMD disease in Chandauli district of Uttar Pradesh state, India.

II. Materials and Methods

a) Study area, animal and management

The incidence occurred in village Daina, district Chandauli, Uttar Pradesh, India. At the time of incidence the population of dairy animals (cattle and buffaloes) in the village was approximately 1200. The animals were kept individually or in groups. The animal rearing practices included stall feeding of wheat/paddy straw, concentrate, and mineral mixture with *ad lib* water

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supply. Buffaloes of the village were having access to local water body situated outside the village.

b) History, clinical examination and data collection

During the visit, animals were thoroughly examined by animal health researchers of Department of Veterinary Epidemiology and Preventive Medicine; and Department of Veterinary Clinical Medicine, Uttar Pradesh Pandit Deen Dayal Upadhayaya Pashu Chikitsa Vigyan Vishwavidyalaya Evum Go-Anusandhan Sansthan (DUVASU), Mathura, India. The health and basic record books of the herd, compiled by veterinary and animal care staff, were also examined and analysed for occurrence of the disease, morbidity and mortality etc. All the animals were having the history of vaccination with *Pasteurella multocida* biotype A vaccine (Biological Product Section, Badshabagh, Lucknow). However, FMD vaccination was lacking in the village. The area was having the history of flood or water logging nearly two months prior to outbreak. The hygiene and sanitation conditions in the villages were unsatisfactory. Majority of the animals were suffering with pyrexia, vesicular lesions in teats and foot (Figure 2 and 3) and respiratory distress. The morbidity and mortality rate was quite high with the death of large number of animals. The clinical signs and history were suggestive of Foot-and-Mouth Disease.

Figure 2 : Vesicular lesion on teats

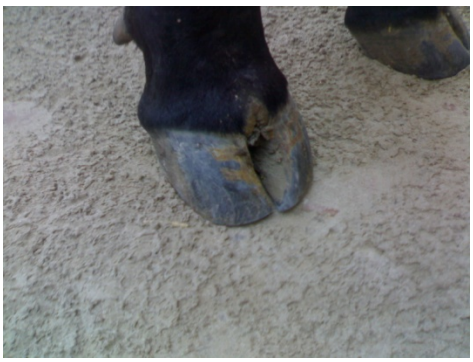

Figure 3 : Vesicular lesions on feet

c) Sample collection

About 15 blood samples from sick animals were collected aseptically from juglar vein and transported on ice to the laboratory. Sera from all the blood samples were separated and stored at 20°C, while clots were processed for the microbiological identification.

d) Laboratory Examination

All the 15 sera samples were analyzed for the presence of antibodies against FMD virus type O, A and Asia-1 using liquid phase blocking ELISA (LPB-ELISA) as per the method described by Hamblin *et al.*, 1986. All the 15 blood clots were processed for bacteriological examination by incubating the samples at 37°C onto Nutrient Agar, MacConkey Agar and 5% Sheep Blood Agar and examined after 24-48h as per the method described by Cruickshank *et al.,* 1975. The causative agent was isolated and characterized with conventional microbiological and biochemical methods.

e) Antibiogram

Pseudomonas aeruginosa isolates were assessed for their antimicrobial susceptibility testing against commonly used antibacterial drugs by disc-diffusion method (Bauer *et al.,* 1996) following the NCCLS guidelines (NCCLS, 2002).

III. Results and Discussion

In the present study, clinical signs of the animals were indicative of Foot-and-Mouth Disease. On retrospective study, the sera samples showed high titre against FMDV serotype 'O' suggesting the infection of serotype 'O' of FMD virus. However, there is as such no report of high mortality due to FMD in cattle and

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particularly in buffaloes. Serotype 'O' is predominantly causes FMD in cattle but in this outbreak high titre against FMDV serotype 'O' in buffaloes also suggest some antigenic alteration or host adaptability of pre-existing serotype 'O' virus in India. There are previous studies reporting the high prevalence of FMDV serotype 'O' in Uttar Pradesh state, India (Verma *et al.,* 2008). The involvement of buffaloes in such outbreaks with higher rate of mortality than cattle is of major concern as buffaloes were suggested to show clinical signs less commonly as compared to cattle (Chakraborty *et al.,* 2014). Moreover, India is having the largest population of buffaloes, accounting for nearly 57% of the world buffalo population, and buffaloes are considered as back bone of rural economy (Kumar, 2005).

Monoculture *Pseudomonas aeruginosa* was isolated from blood. The isolated bacteria were found to be motile, produced characteristics colonies in nutrient agar along with pigmentation, showed β-hemolysis on blood agar (Figure 4) and grew on MacConkey agar.

Figure 4 : β-hemolysis on blood agar

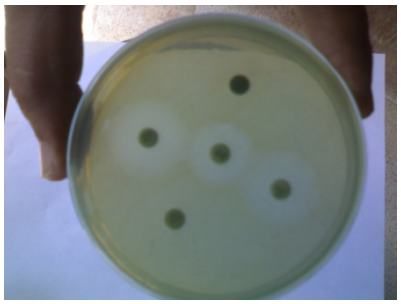

Figure 5 : Antibiogram on Nutrient agar

On Gram's staining, the bacteria appeared Gram-negative, pink colour, medium size bacilli. Biochemical examination revealed battery of reactions characteristic to *Pseudomonas aeruginosa*. Based on the zone of inhibition, observations of drug sensitivity tests revealed resistance against amoxicillin+clavulanic acid, enrofloxacin, ofloxacin, levofloxacin, intermediate sensitivity to penicillin, gentamicin and tylosin, and sensitivity to amikacin, ceftriaxone+tazobactum and cefotaxim (Figure 5). The present findings were comparable to the studies conducted by previous researchers (Sun *et al.,* 2011; Ohnishi *et al.,* 2011; Mudau *et al.,* 2013). On the basis of present findings inference can be drawn that it was an outbreak of FMD, followed by secondary bacterial infection of *Pseudomonas aeruginosa*, which might have entered in the circulation through the lesions in foot, tongue and teats. Moreover, immunosuppression due to FMD further led to colonization of *Pseudomonas aeruginosa* in critical body organs, such as the lungs, heart and kidney leading to severe mortality. On the basis of results of antibiotic sensitivity testing, the authors suggested amikacin, ceftriaxone + tazobactum and cefotaxim for the treatment of affected animals and after treatment with these drugs the mortality among animals was controlled in the village.

IV. Conclusion

It can be concluded that occurrence of FMD leads to immunosuppression making affected animals more susceptible and prone to nosocomial infections viz. *Pseudomonas aeruginosa.* The drug sensitivity pattern revealed that isolates were resistant to many of commonly used broadspectrum antibiotics which might be the cause of failure of treatment. However, antibacterial like amikacin, ceftriaxone + tazobactum and cefotaxim appeared to be drugs of choice for the treatment of *Pseudomonas* infection as the recommendation of these drugs controlled the mortality of animals in the village. Hence, the control of secondary invaders should be considered on priority to avoid the mortality in the outbreak situations.

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Morphology and Morphometry of Indigenous Ducks of Tamil Nadu

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Abstract- Duck production in Tamil Nadu is characterised by the traditional enterprise with indigenous ducks, distributed widely. The indigenous duck varieties of Tamil Nadu have evolved over the years with better adaptability and production potentiality. These indigenous ducks are capable of laying 180-200 eggs per annum with an average egg weight ranging from 60-64 g with no additional or special feeding management. The common Indian breeds/genetic groups of ducks are Indian Runner, Nageswari, Sythetmete, Kuttanad, Arni etc. Besides non-descript ducks are also available in large numbers in many states of the country, contributing significantly to the total duck population. The unique nature of this native germplasm has not been properly documented. Hence, the work was proposed to study morphology and morphometric analysis of distinct indigenous ducks of Tamil Nadu.

The duck farmers in the northern districts of Tamil Nadu are rearing two predominant varieties of ducks i.e. Sanyasi and Keeri. Among these, Sanyasi female is the popular duck variety reared by the farmers. The Sanyasi female ducks are having saffron coloured plumage and males are with dark brown plumage mixed with black.

Keywords: morphology, morphometric traits, indigenous ducks, tamil nadu.

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Morphology and Morphometry of Indigenous Ducks of Tamil Nadu

P.Veeramani [α], R.Prabakaran [σ], S.T.Selvan [ρ], S.N.Sivaselvam [ω] & T.Sivakumar [¥]

Abstract- Duck production in Tamil Nadu is characterised by the traditional enterprise with indigenous ducks, distributed widely. The indigenous duck varieties of Tamil Nadu have evolved over the years with better adaptability and production potentiality. These indigenous ducks are capable of laying 180-200 eggs per annum with an average egg weight ranging from 60-64 g with no additional or special feeding management. The common Indian breeds/genetic groups of ducks are Indian Runner, *Nageswari, Sythetmete, Kuttanad, Arni* etc. Besides non-descript ducks are also available in large numbers in many states of the country, contributing significantly to the total duck population. The unique nature of this native germplasm has not been properly documented. Hence, the work was proposed to study morphology and morphometric analysis of distinct indigenous ducks of Tamil Nadu.

The duck farmers in the northern districts of Tamil Nadu are rearing two predominant varieties of ducks i.e. *Sanyasi* and *Keeri.* Among these, *Sanyasi* female is the popular duck variety reared by the farmers. The *Sanyasi* female ducks are having saffron coloured plumage and males are with dark brown plumage mixed with black. The bill colour of females is orange and for males it is dark yellow. The shank colour is orange for both males and females. The *Keeri* female ducks are having mixture of black and brown plumage characteristically in striations and males are with mixture of dark black and white plumage. The bill colour and shank colour of females is grey. *Keeri* male duck has dark grey / yellow bill colour and oranged coloured shank. Among the recorded morphometric traits, differences were noticed between the sexes and no significant differences were noticed between varieties.

Keywords: morphology, morphometric traits, indigenous ducks, tamil nadu.

I. Introduction

Duck production in India is largely a traditional enterprise and has not yet been industrialized as that of chicken. Even though, duck contributes next to chicken, it is still a neglected species. Being the neglected species for many decades, this native poultry species is threatened for existence due to lack of scientific breeding and management practices. The distribution and demographic dynamics of duck population in India revealed that they are mainly concentrated in eastern, north eastern and southern states of the country. The leading states in duck population are West Bengal, Assam, Kerala, Andhra Pradesh, Tamil Nadu, Bihar and Orissa. The common Indian breeds/genetic groups of ducks are Indian Runner, *Nageswari, Sythetmete, Kuttanad, Arni* etc. In Tamil Nadu, 70 per cent of the duck population is concentrated in six districts namely, Kancheepuram, Thiruvallur, Villupuram, Cuddalore, Vellore and Thiruvannamalai, falling under northern agro-climatic zone of Tamil Nadu (Sivakumar *et al.,* 2009). Existence of different indigenous duck varieties namely Arni, *Sanyasi* and *Keeri*

(Gajendran and Karthickeyan, 2009; Murugan et al., 2009; Veeramani et al., 2009;) with distinct phenotypic characters and better production potential in northern districts of Tamil Nadu were reported. These indigenous ducks have innate potential to produce eggs and meat at considerable quantity with lesser input.

So far, there is no guided breeding and scientific management practices followed in the country, which would lead to loss of the rich native duck germplasm. There is lack of sufficient scientific information on ducks, either phenotypic or genotypic to differentiate various duck breeds or distinct varieties. The duck germplasm is not properly utilized due to various difficulties in duck rearing in the rural environment. Hence, the work was proposed to study phenotypic character and morphometric analysis of this distinct indigenous ducks of Tamil Nadu.

II. Materials and Methods

The morphology of indigenous ducks was studied as per the breed descriptors of the Food and Agriculture Organization (FAO, 1986) and the guidelines given by the National Bureau of Animal Genetic Resources, Karnal, Haryana, India. The morphological characters studied were plumage pattern, carriage, bill colour and shank colour.

III. Morphometry

Body measurements were taken for ducks of *Sanyasi* and *Keeri* variety with a standard measuring tape to the nearest 0.1 centimetre (cm) for bill length, shank length, neck length and body length. The data collected were scrutinized, edited and analysed as per standard statistical procedures (Snedecor and Cochran., 1989).

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IV. Results and Discussion

The duck farmers in the northern districts of Tamil Nadu are rearing two predominant duck varieties i.e. *Sanyasi* and *Keeri*. Among these, *Sanyasi* female is the popular duck variety being reared by the farmers. Each variety is having different phenotypic character.

V. Morphology

The *Sanyasi* female ducks are having saffron coloured plumage with or without white ring like feathers around the neck and males are with dark brown plumage mixed with black. The head and neck covered with lustrous brown plumage. Males have brown coloured drake feather. The bill colour of females is orange and for males it is yellowish orange. The shank colour is orange for both males and females.

The *Keeri* female ducks are having mixture of black and brown plumage characteristically in striations with or without white ring like feathers around the neck and males are with mixture of dark black and white plumage. The head and neck covered with lustrous black plumage. The bill colour and shank colour of females is grey / orange. *Keeri* male duck has dark yellow bill colour and oranged coloured shank. The drake feather is black in colour. Similar plumage pattern, bill colour and shank colour was observed by Murugan et al., (2009).

VI. Morphometric Traits

The morphometric traits such as, body length, neck length, bill length and shank length were recorded for 909 adult ducks comprising of 488 *Sanyasi* and 421 *Keeri* varieties of ducks. The least square means with S.E. is presented in Table-1.

Table 1 : Least-squares mean (± S.E.) of morphometric traits of indigenous ducks of Tamil Nadu

Particulars	Number of observations	Body length (cm)	Neck length (cm)	Bill length (cm)	Shank length (cm)
Overall mean	**909**	**23.74±0.06**	**13.19±0.17**	**6.30±0.01**	**5.58±0.01**
Variety		**NS**	**NS**	**NS**	**NS**
Sanyasi	488	23.85±0.09	13.47±0.25	6.29±0.01	5.58±0.02
Keeri	421	23.64±0.08	12.90±0.22	6.31±0.01	5.59±0.02
Sex		**	**	**	**
Male	201	24.53±0.11	13.94±0.29	6.84±0.02	5.61±0.02
Female	708	22.95±0.06	12.43±0.15	5.76±0.01	5.56±0.01
Sex X Variety		**NS**	**NS**	**NS**	**NS**
Male					
Sanyasi	81	24.74±0.17	13.50±0.46	6.82±0.02	5.59±0.03
Keeri	120	24.33±0.13	13.38±0.37	6.87±0.01	5.65±0.04
Female					
Sanyasi	407	22.96±0.07	12.45±0.20	5.75±0.02	5.57±0.02
Keeri	301	22.94±0.09	12.42±0.23	5.77±0.01	5.56±0.02

*NS- Non-significant (P≤0.05); * - Significant (P≤ 0.05); ** - Significant (P≤0.01)*

VII. Body Length

The overall body length for both the varieties recorded was 23.74±0.06 cm. Body length for *Sanyasi* and *Keeri* varieties was 23.85±0.09 and 23.64±0.08 cm respectively. The numerical difference in body length between varieties was not statistically significant. The value for male and female ducks was 24.53±0.11 and 22.95±0.06 cm respectively and the difference between the sexes was highly significant (P<0.01). On the contrary, Yakubu (2009) recorded mean values of body length (cm) for male and female African Muscovy ducks as 47.86 and 38.35. The lower valued obtained in this study might be due to the variation in the size and conformation of the distinct variety / breed of ducks.

VIII. Neck Length

The neck length recorded for *Sanyasi* and *Keeri* varieties was 13.47±0.25 and 12.90±0.22 respectively with overall neck length of 13.19±0.17 cm. Among the sexes the difference in neck length was highly significant (P<0.01). The value for male and female adult ducks was 13.94±0.29 and 12.43±0.15 respectively. The interaction between variety and sex had no significant effect on neck length. whereas, Yakubu (2009) recorded the mean neck length for male and female African Muscovy ducks as 18.10 and 14.33 cm respectively, while Murugan et al. (2009) recorded the neck length (cm) of 21.10 ± 0.12and 18.70 ± 0.24for male and female *Sanyasi* ducks respectively. The neck length of

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20.23 ± 0.14and 17.15 ± 0.45cm was recorded for male and female ducks of *Keeri* varieties. The difference in neck length might be due to breed variation and errors in measurement.

IX. Bill Length

The recorded bill length for male ducks of *Sanyasi* and *Keeri* varieties was 6.82±0.02 and 6.87±0.01 cm respectively, while the value for female ducks was 5.75±0.02 and 5.77±0.01 cm respectively. Within the sex the variety had no significant effect on bill length. The bill length for male and female ducks recorded was 6.84±0.02 and 5.76±0.01 cm respectively. This revealed a highly significant variation among the sexes. The overall bill length for two varieties of ducks was 6.30±0.01 cm. Similarly, Ajith et al. (2009) recorded significantly higher bill length in males in comparison with respective females with regard to *Chara* and *Chemballi* ducks of Kerala. Whereas, shorter bill length of 4.98 and 3.75 cm was recorded for African Muscovy male and female ducks by Yakubu (2009). The bill length for *Sanyasi* and *Keeri* ducks of Tamil Nadu was recorded by Murugan et al. (2009), which is in comparison with the values of this present study. The higher value of bill length in males than female ducks might be attributed to their heavier size and adaptability.

X. Shank Length

Significantly higher shank length for males than female ducks was recorded in both the varieties (5.61±0.02 cm for males and 5.56±0.01 cm for females), on the other hand, variety had no significant role on shank length (5.58±0.02 cm for *Sanyasi* and 5.59±0.02 cm for *Keeri* variety). The overall shank length was 5.58±0.01 cm. The interaction between sex and variety also had no significant effect on shank length. While, Renchi et al. (1979) recorded the mean shank length in male and female Desi ducks of Kerala at 12 weeks of age as 6.44±0.04 and 6.15±0.02 cm and reported that males had significantly higher shank length than female ducks and similar values were recorded by Ajith et al. (2009) for *Chara* and *Chemballi* ducks of Kerala. Whereas, in *Nageswari* ducks of Assam, Zaman et al. (2007) recorded the mean shank length of male and female as 6.67±0.71 and 6.12±0.68 cm respectively. The difference in the shank length of different varieties of indigenous ducks might be attributed to the variation among indigenous germplasm and adaptability to the rearing environment.

XI. Conclusion

The existence of two distinct indigenous duck varieties namely *Sanyasi* and *Keeri* was fully evidenced through this study. These varieties were having different morphology and morphometry with other indigenous duck varieties of the country. Since, the concept of rearing breeder flock and proper selection among the duck varieties are the biggest lacunas in the study area, necessary steps to address these constraints will throw more light on these unique germplasm. Moreover, these duck germplasm are known for its prolificacy under nil-input system of management, further studies focusing on large scale survey, characterisation of these ducks at molecular level will be the best approach for proper selection and conservation of these unique germplasm for future use and exploitation.

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Sanyasi Ducks

Male

Female

Keeri Ducks

Male

Female

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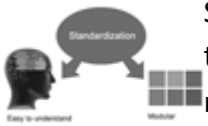

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3. Submission of Manuscripts,

4. Manuscript's Category,

5. Structure and Format of Manuscript,

6. After Acceptance.

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- Submitting a manuscript with pages out of sequence

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· Use standard writing style including articles ("a", "the," etc.)

· Keep on paying attention on the research topic of the paper

· Use paragraphs to split each significant point (excluding for the abstract)

· Align the primary line of each section

· Present your points in sound order

· Use present tense to report well accepted

· Use past tense to describe specific results

· Shun familiar wording, don't address the reviewer directly, and don't use slang, slang language, or superlatives

· Shun use of extra pictures - include only those figures essential to presenting results

Title Page:

Choose a revealing title. It should be short. It should not have non-standard acronyms or abbreviations. It should not exceed two printed lines. It should include the name(s) and address (es) of all authors.

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Abstract:

The summary should be two hundred words or less. It should briefly and clearly explain the key findings reported in the manuscript--must have precise statistics. It should not have abnormal acronyms or abbreviations. It should be logical in itself. Shun citing references at this point.

An abstract is a brief distinct paragraph summary of finished work or work in development. In a minute or less a reviewer can be taught the foundation behind the study, common approach to the problem, relevant results, and significant conclusions or new questions.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Yet, use comprehensive sentences and do not let go readability for briefness. You can maintain it succinct by phrasing sentences so that they provide more than lone rationale. The author can at this moment go straight to shortening the outcome. Sum up the study, with the subsequent elements in any summary. Try to maintain the initial two items to no more than one ruling each.

- Reason of the study - theory, overall issue, purpose
- Fundamental goal
- To the point depiction of the research
- Consequences, including definite statistics - if the consequences are quantitative in nature, account quantitative data; results of any numerical analysis should be reported
- Significant conclusions or questions that track from the research(es)

Approach:

- Single section, and succinct
- As a outline of job done, it is always written in past tense
- A conceptual should situate on its own, and not submit to any other part of the paper such as a form or table
- Center on shortening results - bound background information to a verdict or two, if completely necessary
- What you account in an conceptual must be regular with what you reported in the manuscript
- Exact spelling, clearness of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else

Introduction:

The **Introduction** should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable to comprehend and calculate the purpose of your study without having to submit to other works. The basis for the study should be offered. Give most important references but shun difficult to make a comprehensive appraisal of the topic. In the introduction, describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will have no attention in your result. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here. Following approach can create a valuable beginning:

- Explain the value (significance) of the study
- Shield the model - why did you employ this particular system or method? What is its compensation? You strength remark on its appropriateness from a abstract point of vision as well as point out sensible reasons for using it.
- Present a justification. Status your particular theory (es) or aim(s), and describe the logic that led you to choose them.
- Very for a short time explain the tentative propose and how it skilled the declared objectives.

Approach:

- Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done.
- Sort out your thoughts; manufacture one key point with every section. If you make the four points listed above, you will need a least of four paragraphs.

- Present surroundings information only as desirable in order hold up a situation. The reviewer does not desire to read the whole thing you know about a topic.
- Shape the theory/purpose specifically - do not take a broad view.
- As always, give awareness to spelling, simplicity and correctness of sentences and phrases.

Procedures (Methods and Materials):

This part is supposed to be the easiest to carve if you have good skills. A sound written Procedures segment allows a capable scientist to replacement your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt for the least amount of information that would permit another capable scientist to spare your outcome but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section. When a technique is used that has been well described in another object, mention the specific item describing a way but draw the basic principle while stating the situation. The purpose is to text all particular resources and broad procedures, so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step by step report of the whole thing you did, nor is a methods section a set of orders.

Materials:

- Explain materials individually only if the study is so complex that it saves liberty this way.
- Embrace particular materials, and any tools or provisions that are not frequently found in laboratories.
- Do not take in frequently found.
- If use of a definite type of tools.
- Materials may be reported in a part section or else they may be recognized along with your measures.

Methods:

- Report the method (not particulars of each process that engaged the same methodology)
- Describe the method entirely
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures
- Simplify - details how procedures were completed not how they were exclusively performed on a particular day.
- If well known procedures were used, account the procedure by name, possibly with reference, and that's all.

Approach:

- It is embarrassed or not possible to use vigorous voice when documenting methods with no using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result when script up the methods most authors use third person passive voice.
- Use standard style in this and in every other part of the paper - avoid familiar lists, and use full sentences.

What to keep away from

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings - save it for the argument.
- Leave out information that is immaterial to a third party.

Results:

The principle of a results segment is to present and demonstrate your conclusion. Create this part a entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Carry on to be to the point, by means of statistics and tables, if suitable, to present consequences most efficiently.You must obviously differentiate material that would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matter should not be submitted at all except requested by the instructor.

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Content

- Sum up your conclusion in text and demonstrate them, if suitable, with figures and tables.
- In manuscript, explain each of your consequences, point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation an exacting study.
- Explain results of control experiments and comprise remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or in manuscript form.

What to stay away from

- Do not discuss or infer your outcome, report surroundings information, or try to explain anything.
- Not at all, take in raw data or intermediate calculations in a research manuscript.
- Do not present the similar data more than once.
- Manuscript should complement any figures or tables, not duplicate the identical information.
- Never confuse figures with tables - there is a difference.

Approach

- As forever, use past tense when you submit to your results, and put the whole thing in a reasonable order.
- Put figures and tables, appropriately numbered, in order at the end of the report
- If you desire, you may place your figures and tables properly within the text of your results part.

Figures and tables

- If you put figures and tables at the end of the details, make certain that they are visibly distinguished from any attach appendix materials, such as raw facts
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- In spite of position, each table must be titled, numbered one after the other and complete with heading
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Discussion:

The Discussion is expected the trickiest segment to write and describe. A lot of papers submitted for journal are discarded based on problems with the Discussion. There is no head of state for how long a argument should be. Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implication of the study. The purpose here is to offer an understanding of your results and hold up for all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of result should be visibly described. Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved with prospect, and let it drop at that.

- Make a decision if each premise is supported, discarded, or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."
- Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work
- You may propose future guidelines, such as how the experiment might be personalized to accomplish a new idea.
- Give details all of your remarks as much as possible, focus on mechanisms.
- Make a decision if the tentative design sufficiently addressed the theory, and whether or not it was correctly restricted.
- Try to present substitute explanations if sensible alternatives be present.
- One research will not counter an overall question, so maintain the large picture in mind, where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

- When you refer to information, differentiate data generated by your own studies from available information
- Submit to work done by specific persons (including you) in past tense.
- Submit to generally acknowledged facts and main beliefs in present tense.

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	A-B	**C-D**	**E-F**
Abstract	Clear and concise with appropriate content, Correct format. 200 words or below	Unclear summary and no specific data, Incorrect form Above 200 words	No specific data with ambiguous information Above 250 words
Introduction	Containing all background details with clear goal and appropriate details, flow specification, no grammar and spelling mistake, well organized sentence and paragraph, reference cited	Unclear and confusing data, appropriate format, grammar and spelling errors with unorganized matter	Out of place depth and content, hazy format
Methods and Procedures	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
Result	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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