IMPACT OF OVULATION SYNCHRONIZATION PROTOCOLS IN ADDRESSING INFERTILITY OF CROSSBRED DAIRY CATTLE

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ABSTRACT

Comparative efficacy of six estrus/ovulation synchronization protocols, viz., Ovsynch, Ovsynch plus, PRID/Triu-B, PRID+PMSG, Doublesynch and Estradoublesynch was studied for their estrus induction response, and conception rates at FTAI and overall of three cycles post-treatment including monitoring of plasma progesterone (P_4) at different time intervals in postpartum anestrus and repeat breeding Holstein crossbred cattle (17-36 animals per protocol) under field conditions. Induction and synchronization of estrus with establishment of normal cyclicity was found in 100 per cent anestrus animals with PRID, PRID+PMSG, Doublesynch, Estradoublesynch protocols, but the response with Ovsynch & Ovsynch plus was 92 and 87 per cent, respectively. The conception rates obtained at induced estrus/FTAI in anestrus cattle under Ovsynch, Ovsynch plus, PRID, PRID+PMSG, Doublesynch protocols were 36.4, 10.0, 30.0, 40.0, 37.1 and 19.2 per cent (p<0.01), respectively, with overall 3 cycles' conception rates of 60.6, 45.0, 60.0, 70.0, 60.0 and 38.5 per cent (p<0.05), respectively, post-treatment. In repeat breeder cattle, the conception rates obtained at induced estrus/FTAI with Ovsynch, Ovsynch plus, Doublesynch and Estradoublesynch protocols were 34.8, 23.5, 34.6 and 20.0 per cent (p<0.01), respectively, and overall 3 cycles' conception rates were 60.9, 47.1, 57.7 and 45.0 per cent (p<0.01), respectively. Among untreated anestrus and repeat breeding control animals only 20.0 per cent animals each conceived over 60-90 days follow up. The plasma P_4 was significantly (p<0.05) higher in conceived than non-conceived animals on day 12 post-AI in all the groups suggesting anovulatory induced estrus and/or luteal insufficiency in non-conceived animals. Thus, it was concluded that the progestagen based protocols were ideal in anestrus cows followed by Ovsynch or Doublesynch protocol in both anestrus and repeat breeders to economically improve the conception rates.

Keywords: Anestrus, Conception rate, Crossbred cows, Estrus synchronization, Plasma progesterone, Repeat breeder.

Postpartum anestrus and repeat breeding are the major infertility issues in dairy animals causing great economic loss to the animal breeders and to the dairy industry (Bhoraniya et al., 2012; Dhami et al., 2015). Effective treatment of infertile cows is the efficient means of improving their reproductive and productive potential. Different hormonal preparations and protocols are being used to modulate the circulating plasma progesterone levels to make anestrus and repeat breeding animals to cycle normally and to improve their reproductive efficiency, thereby reducing the inter-calving interval economically viable (Abubaker et al., 2013; Dhami et al., 2015; Chaudhary et al., 2018). But the results reported in the literature are inconsistent (Randel et al., 1996; Khade et al., 2011; Patel et al., 2013; Prajapati et al., 2018) largely due to nutritional status, faulty management, ovarian changes, endocrine events and even uterine infection. Hormonal therapies have good therapeutic value to enhance reproductive efficacy only in infection free infertile animals with good nutritional status (Bhoraniya et al., 2012; Buhecha et al., 2015; Chaudhary et al., 2018). Very little information is available on use of recently developed Doublesynch, Estradoublesynch and PRID+PMSG protocols on cyclic/acyclic cattle with respect to conception rate and plasma progesterone under field condition. Hence, present study was planned to evaluate the comparative efficacy of different estrus induction and ovulation synchronization protocols through monitoring plasma progesterone and conception rates in anestrus and repeat breeding crossbred cows under field conditions.

MATERIALS AND METHODS

Selection of infertile animals

The study was carried out over two years (2015-17) in villages under the milk shed areas of Amul, Anand and Panchamrut Dairy, Godhra, Gujarat, India. The postpartum true anestrus (>90 days acyclic) and classical repeat breeding (>140 days cyclic) crossbred cows of average Body condition score (BCS) were screened gynaecoclinically for their reproductive status. Anestrus animals were confirmed by palpating inactive ovaries per rectum twice 10 days apart. Repeat breeders were confirmed on the basis of regular cyclicity and AI for more than 3 times without visible or palpable genital abnormality, yet failed to conceive.

Pre-synchronization treatment

All the infertile animals selected were initially injected once with 100 mg Ivermectin s/c (Inj. Ivectin, 10 ml, Indian Immunologicals Ltd), and injection organic phosphorus (Inj. Tonophosphan, MSD Animal Health) and multivitamin (Inj. Intavita-H, Intas Pharma) 10 ml each, and bolus Minotas (Intas Pharma) @ one bolus PO for 7 days. Repeat breeder animals received additional single shot i/m injection of Enrofloxacin (Inj. Flobac SA, 40 ml, Intas Pharma) to rule out invisible genital infection, if any. The cows were then randomly subjected to following six estrus induction/ synchronization protocols, keeping one group each of untreated acyclic and cyclic controls. Progestagen based PRID (Triu-B) and PRID + PMSG protocols were used only in anestrus cows.

Ovsynch and Ovsynch Plus protocols

Under Ovsynch protocol, the animals were

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PRID + PMSG Protocol

administered with 20 μ g GnRH analogue, i.e. Buserelin acetate (Receptal, 5 ml, MSD) on day 0, 500 μ g PGF₂ α analogue, i.e. Cloprostenol sodium (Estrumate, 2 ml, MSD) on day 7, and again 10 μ g GnRH on day 9, intramuscularly followed by fixed time AI (FTAI) twice at 16 and 24 hrs later. In Ovsynch Plus protocol, cows received an additional i/m Inj. of PMSG 500 IU (Folligon, MSD) 2 days before the actual Ovsynch protocol.

Doublesynch and Estradoublesynch protocols

Under Doublesynch protocol, the cows were administered intramuscularly with 500 μ g Cloprostenol sodium on day 0, 20 μ g Buserelin acetate on day 2, and again 500 μ g Cloprostenol on day 9 and 10 μ g Buserelin acetate on day 11, followed by FTAI twice at 16 and 24 hrs later, while in Estradoublesynch protocol, the infertile cows received an Inj. of estradiol benzoate 1 mg (Sigma, USA) on day 10, in place of second GnRH injection on day 11 in Doublesynch, with FTAI twice at 48 and 60 hrs postestradiol injection.

PRID and PRID Plus PMSG protocols

Under PRID protocol, Triu-B (0.96 g progesterone in elastic rubber molded over a nylon spine, Virbac Animal Health Pvt Ltd) was inserted intra-vaginally for 7 days in anestrus cows. On the sixth day, the animals were treated with i/m injection of 500 μ g Cloprostenol sodium. Estradiol benzoate-EB 0.75 mg was given i/m on day 7 while removing the vaginal insert. FTAI was done 24 hrs post-EB treatment. In PRID Plus PMSG protocol, an Inj. of PMSG 500 IU (Folligon, MSD) replaced the estradiol



benzoate in PRID protocol, and FTAI was done twice at 48 and 60 hrs letter.

Control groups

Ten anestrus and 10 repeat breeder cows each given pre-synchronization treatment as above, without any hormonal intervention and followed for spontaneous estrus and insemination, served as untreated controls. Following FTAI, visual heat detection for half an hour both in the morning and evening and frozen semen inseminations were practiced for two more cycles, if the animal failed to conceive at FTAI. In non-return cases pregnancy was confirmed per-rectum 60 days of last AI. The observations on estrus responses and conception rates were recorded for all groups.

Blood sampling and progesterone Assay

Blood samples were collected from jugular vein of 12 representative animals of each treatment protocol in heparinized vaccutainers, on day 0- just before initiation of treatment, on day 6/7/9/10- at the time of PGF₂ α injection, on day 8/9/10/12- at induced estrus/FTAI (as per protocol) and on day 12 post-AI. The blood samples were centrifuged at 3000 rpm for 15 minutes, and plasma separated out was stored at -20°C with a drop of merthiolate (0.1%). Plasma progesterone concentrations were estimated by employing standard Radio-Immuno-Assay (RIA) technique of Kubasic *et al.* (1984). Labelled antigen (I¹²⁵), antibody coated tubes and standards were procured from Immunotech-SAS, France.

Statistical analysis

The data on estrus response and conception rates between different groups were statistically analysed using Chi-square test and those of progesterone profile by using ANOVA and DMRT (Snedecor and Cochran, 1986).

RESULTS AND DISCUSSION

Estrus synchronization and fertility

The estrus induction responses and conception rates in anestrus and repeat breeding cows under different protocols and under control groups are presented in Tables 1 and 2. Among the true anestrus cows each subjected to Ovsynch (n=36) and Ovsynch plus (n=23) estrus induction response was 92 and 87 per cent, respectively, whereas, in Doublesynch (n=35), Estradoublesynch (n=26), PRID/Triu-B (n=20) and PRID+PMSG (n=20)

Table 1:							
Efficacy of different	t estrus syno	chronization protocols	in terms of estrus induct	ion response and conc	eption rates in anesti	rus cows	
	$\begin{array}{c} \text{of different estrus synchromody} \\ \text{zation} \\ \text{ol} \\ & \text{Orbit} \\ \text{Cow} \\ \hline \\ & 36 \\ \text{Cow} \\ \hline \\ & 23 \\ \text{S} \\ & 35 \\ \text{ynch} \\ 26 \\ 20 \\ \text{S} \\ & 20 \\ \text{ntrol} \\ 10 \\ \hline \end{array}$	% Estrus _ Induction Response	Conception Rate (%)				
Synchronization Protocol			Induced 1 st estrus	2 nd oestrus cycle	3 rd oestrus cycle	Overall of 3 oestrus	
Ovsynch	36	91.7 (33/36)	36.4 (12/33)	31.6 (6/19)	20.0 (2/10)	60.6 (20/33)	
Ovsynch plus	23	87.0 (20/23)	10.0 (2/20)	29.4 (5/17)	20.0 (2/10)	45.0 (9/20)	
Doublesynch	35	100	37.1 (13/35)	28.6 (6/21)	15.4 (2/13)	60.0 (21/35)	
EstraDoublesynch	26	100	19.2 (5/26)	15.8 (3/19)	16.7 (2/12)	38.5 (10/26)	
PRID/Triu-B	20	100	30.0 (6/20)	41.6 (5/12)	16.7 (1/6)	60.0 (12/20)	
PRID+PMSG	20	100	40.0 (8/20)	36.4 (4/11)	33.3 (2/6)	70.0 (14/20)	
Anoestrus control	10	20 (2/10)	50.0 (1/2)	100.0 (1/1)	0	20.0 (2/10)	
χ^2 Values for			24.26	12.37	10.40	11.87	
treatment groups	-	-	(p<0.01)	(p<0.05)	NS	(p<0.05)	

Table 2:

Validation of different estrus synchronization protocols in terms of estrus induction response and conception rates in repeat breeder cows

Synchronization Protocol	No.	% Estrus Induction Response	Conception Rate (%)				
	of Cow		Induced 1 st estrus	2 nd oestrus cycle	3 rd oestrus cycle	Overall of 3 oestrus cycles	
Ovsynch	23	100	34.8 (8/23)	25.0 (4/16)	18.2 (2/11)	60.9 (14/23)	
Ovsynch plus	17	100	23.5 (4/17)	25.0 (3/12)	12.5 (1/8)	47.1 (8/17)	
Doublesynch	26	100	34.6 (9/26)	31.3 (5/16)	10.0 (1/10)	57.7 (15/26)	
EstraDoublesynch	20	100	20.0 (4/20)	25.0 (4/16)	09.1 (1/11)	45.0 (9/20)	
RB control	10	100	10.0 (1/10)	11.1 (1/9)	0	20.0 (2/10)	
χ^2 Values for			18.18	26.83	3.92	22.66	
treatment groups	-	-	(p< 0.01)	(p< 0.01)	NS	(p < 0.01)	

Table 3:

Plasma progesterone profile on different days of synchronization and on day 12 post-AI in anestrus and repeat breeder cows

S			Plasma progesterone (ng/ml)				
tatu	Synchronization	No.	Initiation of	Day of PG Inj.	Day of FTAI	D-12 post-	
S	protocol		Treatment (D-0)	(D-6/7/9/10)	(D-8/ 9/10/12)	AI	
Anestrus	Ovsynch	12	0.41 ± 0.11^{a}	1.45 ± 0.71^{b}	0.24 ± 0.12^{a}	2.21 ± 0.98^{b}	
	Ovsynch Plus	12	0.22 ± 0.14^{a}	2.75 ± 0.88^{b}	0.14 ± 0.08^{a}	4.27 ± 1.22^{b}	
	Doublesynch	12	0.24 ± 0.07^{a}	0.96 ± 0.59^{b}	0.11 ± 0.05^{a}	1.81 ± 1.02^{b}	
	EstraDoublesynch	12	0.28 ± 0.10^{a}	1.06 ± 0.59^{b}	0.17 ± 0.06^{a}	$2.28\pm0.93^{\circ}$	
	PRID	12	0.26 ± 0.12^{a}	2.88±0.21 ^b	0.15 ± 0.02^{a}	3.18 ± 0.84^{b}	
	PRID+PMSG	12	0.32 ± 0.09^{a}	2.54 ± 0.33^{b}	0.25 ± 0.03^{a}	5.39±0.71°	
Repeaters	Ovsynch	12	1.47 ± 0.31^{b}	2.65 ± 0.79^{bc}	0.29 ± 0.09^{a}	$3.78 \pm 1.08^{\circ}$	
	Ovsynch Plus	12	1.12 ± 0.24^{b}	1.98 ± 0.69^{bc}	0.14 ± 0.08^{a}	$2.27 \pm 1.02^{\circ}$	
	Doublesynch	12	2.68 ± 0.39^{b}	2.78 ± 0.97^{b}	0.11 ± 0.03^{a}	2.33±1.03 ^b	
	EstraDoublesynch	12	1.04 ± 0.12^{b}	3.74±1.02 ^c	0.23 ± 0.05^{a}	1.58 ± 0.89^{b}	

Means bearing superscript in common with the row do not differ significantly (p>0.05).

protocols treated anestrus cows, 100 per cent cows 70.0 per cent (p<0.05), respectively. exhibited synchronized estrus with excellent behavioural signs. Following FTAI in these estrus exhibited cows from Ovsynch, Ovsynch plusDoublesynch, Estradoublesynch, PRID/Triu-B and PRID+PMSG protocols, average conception rate at induced estrus was observed to be 36.4, 10.0, 37.1, 19.2, 30.0 and 40.0 per cent (p<0.01), with an

Among untreated anestrus control group (n=10), 2 out of 10 cows (20 per cent) exhibited estrus over a period of 90 days follow up with a conception rate of 50.0 per cent on AI at first estrus. The conception rates achieved with progestagen based protocols were the highest at induced estrus and overall of 3 cycles', followed by Ovsynch and overall conception rate of 60.6, 45.0, 60.0, 38.5, 60.0 and Doublesynch protocol in anestrus cows, but the results with



Estradoublesynch and Ovsynch plus protocols were significantly lower (Table 1).

Among the repeat breeder cows treated with Ovsynch (n=23), Ovsynch plus (n=17), Doublesynch (n=26) and Estradoublesynch (n=20) protocols, estrus induction rate was 100 per cent with a average conception rate of 34.8, 23.5, 34.6 and 20.0 per cent (p<0.01), respectively, following FTAIs at induced estrus. In these treatment groups, as shown in Table 2, following AI in subsequent 2 oestrus cycles, overall conception rates of 60.9, 47.1, 57.7 and 45.0 per cent (p<0.01), respectively, was obtained in Ovsynch, Ovsynch plus, Doublesynch and Estradoublesynch protocol treated repeat breeder cows.

Among untreated control group of repeat breeder cows, all animals continued to cycle and 2 out of 10 cows (20 per cent) conceived within 2-3 repeated cycles over 60 days follow up. Here also the conception rates achieved at induced estrus and overall of 3 cycles with Ovsynch and Doublesynch were significantly (p<0.01) superior over Ovsynch Plus and Estradoublesynch protocols (Table 2).

The estrus response of 92 and 87 per cent achieved with Ovsynch and Ovsynch Plus protocols, respectively, in anestrus cows was in harmony with the result of 93.7 per cent reported by Virmani et al. (2013) in Sahiwal cows. However, many other researchers (Khade et al., 2011; Ammu et al., 2012; Bhoraniya et al., 2012; Dhami et al., 2015; Borkhatariya et al., 2017; Prajapati et al., 2018a,b) have documented 100 per cent estrus response with either of these protocols in anestrus and/or repeat breeding cattle. The estrus response of 100 per cent achieved with Doublesynch and Estradoublesynch protocols in both anestrus and repeat breeding cows was in harmony with the earlier results of Prajapati et al. (2018a,b), however Chaudhary et al. (2018) and Patel et al. (2018), found 85-95 per cent response in anestrus Gir heifers and buffaloes using same protocols. Further, the 100 per cent estrus response obtained with PRID and PRID+PMSG protocols was in close agreement with the reports of Bhoraniya et al. (2012), Hadiya et al. (2015), Borkhatariya et al. (2017) and Patel et al. (2018) with either of the protocol in anestrus cows.

The present conception rates of 36.4 and 34.8 per cent obtained at induced estrus in acyclic and cyclic cows



with Ovsynch protocol were in range of 30.0 to 41.6 per cent reported by Abubaker *et al.* (2013), Buhecha *et al.* (2015), Hadiya et al. (2015), Parmar et al. (2015) and Prajapati et al. (2018a,b) in acyclic and cyclic cows. Further, the present 60 per cent overall three cycles' conception rates obtained in both anestrus and repeat breeder cattle were in line with 58.3 to 70.0 per cent reported by Khade et al. (2011), Buhecha et al. (2015), Parmar et al. (2015) and Prajapati *et al.* (2018a,b) with the same protocol in cyclic and anestrus cows. However, higher overall conception rates of 73-89 per cent in acyclic and/or cyclic cows were reported by others (Tenhagen et al., 2004; Ammu et al., 2012; Hadiya et al., 2015; Borkhatariya et al., 2017). The conception rates of 10.0 and 23.5 per cent obtained with Ovsynch plus protocol at induced estrus and 45.0 and 47.1 per cent for overall 3 cycles' in anestrus and repeat breeding cows closely concurred with the earlier conception rates of 20-28 per cent at FTAI reported by Virmani et al. (2013) and Prajapati et al. (2018_{ab}) in anestrus and repeat breeding cattle, but were quite lower than 53.3-75.0 per cent reported in anestrus buffaloes by Kumar *et al.* (2016).

The first service conception rates of 37.1 and 34.6 per cent obtained with Doublesynch protocol in anestrus and repeat breeder cows are little lower than earlier reports of 40.0 per cent by Prajapati et al. (2018a,b) in cattle and 55-62 per cent by Mirmahmoudi et al. (2014) and Dhinsa et al. (2016) in anestrus buffaloes. The present 60.0 and 57.7 per cent overall three cycles' conception rates found in acyclic and cyclic cows with Doublesynch protocol closely corroborated with the findings of Chaudhary et al. (2018) in Gir heifers and Patel et al. (2018) in buffaloes. However, Ozturk et al. (2010) and Prajapati et al. (2018a,b) recorded overall conception rates of 72-80 per cent in acyclic and cyclic cows with this protocol. Moreover, the conception rates of 19.2 and 20.0 per cent obtained in acyclic and cyclic animals at induced estrus with Estradoublesynch protocol were in line with earlier results of 20-30 per cent by Roodbari *et al.* (2015) and Prajapati *et al.* (2018a,b), while Mirmahmoudi et al. (2014) found it as 60-64 per cent in buffaloes. Further, the present 38.5 and 45.0 per cent overall three cycles' conception rates with this protocol were in line with Chaudhary et al. (2018), but lower than the earlier finding of 60.0 per cent in acyclic and repeat

breeder cattle (Sharvanan et al., 2016; Prajapati et al. (2018a,b).

The first service and overall three cycles' conception rates of 30 and 60 per cent obtained with PRID in anestrus cows were in close agreement with the findings of Khade et al. (2011), Buhecha et al. (2015) and Borkhatariya et al. (2017), while Bhoraniya et al. (2012), Ammu et al. (2012), Hadiya et al. (2015) obtained 80-100 per cent overall three cycles conception rate in anestrus cows. Further, the first service and overall three cycles' conception rates of 40 and 70 per cent achieved with PRID+PMSG protocol were much in line with reports of Carvalho et al. (2013) and Patel et al. (2018) in anestrus buffaloes. The better results with PRID and PRID+PMSG probably were due to longer and better negative feedback of progesterone on hypothalamus and anterior pituitary gland, and also due to the fact that the $PGF_2\alpha$ increases pituitary responsiveness in postpartum cows (Randel et al., 1996) and hence the released GnRH after CIDR/PRID removal effectively stimulates the pituitary gonadotrophins with subsequent estrus induction (Zaabel et al., 2009). The combination of progesterone with PMSG causes increased estradiol production leading to behavioural estrus followed by ovulation and thus favours conception (Carvalho et al., 2013; Chaudhary et al., 2018).

Progesterone profile

The mean plasma progesterone concentrations in anestrus cows under all protocols were significantly (p<0.05) higher on day 7-10 of protocol (day of PGF₂ α injection) and day 12 post-AI compared to day 0 and day of FTAI, while in repeat breeders the concentrations on day 0 were also significantly higher compared to day of FTAI (Table 3). These results confirmed the reproductive status of two categories of animals at the start of treatment. The higher mean plasma progesterone concentrations in PRID+PMSG protocol on day 12 post-AI as compared to different days of treatment might be due to more number of animals conceived in this group. Sarvaiya et al. (1991) and Bhoraniya et al. (2012) reported that the conceived buffaloes and cows had progesterone levels more than 3.0 ng/ml between days 12 and 22 post-breeding. Higher mean plasma progesterone level recorded on day 6 of treatment in cows under PRID and PRID+PMSG protocols might be due to continuous release of the exogenous progesterone from the progesterone moulded silastic coil inserted in the anterior vagina. In Doublesynch and Estradoublesynch protocols the rise in mean progesterone levels noted on day 9 might be due to luteinisation of some of the growing follicles and/or ovulation of dominant follicle and formation of CL under the influence of GnRH. In Estradoublesynch protocol, the estradiol benzoate injected on day 10 might also have triggered positive feedback effect of estrogen on hypothalamus and pituitary resulting into ovulatory LH surge and thereby improved conception rate in that group. The trend and levels of plasma progesterone observed

under different protocols in treated cows closely concurred with the previous reports of Bhoraniya *et al.* (2012), Buhecha *et al.* (2015), Parmar *et al.* (2015), Dhami *et al.* (2015), Borkhatariya *et al.* (2017), Chaudhary *et al.* (2016) and Prajapati *et al.* (2018a,b) with use of similar protocols in cyclic and/or acyclic cows.

The conceived animals in both anestrus and repeat breeder category were found to have higher mean plasma progesterone as compared to non-conceived ones only on day 12 post-FTAI, but not at other intervals, in all the protocols with significant (p < 0.05) differences in Ovsynch, Doublesynch, Estradoublesynch, and PRID+PMSG protocols. This may be due to varying number of animals that conceived under different protocols and probable anovulatory induced estrus and/or luteal insufficiency in non-conceived animals. The present findings on plasma progesterone profile with respect to effect of Ovsynch, CIDR, CIDR+PMSG, Doublesynch and Ertradoublesynch protocols closely corroborated with the reports of Khade et al. (2011), Bhoraniya et al. (2012). Nakrani et al. (2015), Buhecha et al. (2015), Dhami et al. (2015), Borkhatariya et al. (2017), Chaudhary et al. (2016) and Prajapati et al. (2018a,b) in cyclic and/or acyclic cows treated with similar protocols. The plasma progesterone assay confirmed the estrus response and conception or anovulatory estrus and/or luteal insufficiency in infertile cows treated with different protocols.

CONCLUSION

The study showed excellent induction & synchronization of estrus with establishment of normal cyclicity with all the protocols in both anestrus and repeat breeder cows. The conception rates achieved with progestagen based protocols were the highest at induced estrus and overall of three cycles in anestrus cows, followed by Ovsynch and Doublesynch protocol in both anestrus and repeat breeders. Ovsynch plus protocol infact suppressed conception rate even with expensive injection of PMSG in place of estradiol in anestrus animals. It is thus concluded that the PRID, Ovsynch or Doublesynch protocol can be used to manage the nutritionally sound infertile cows on large scale to reduce their calving intervals and make them productive in shorter time under field conditions with economic benefit.

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