# Studies On Non-Surgical Recovery and Quality of Eggs in Spontaneously Ovulating Normal and Repeat Breeding Cross-Bred Cows

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## ABSTRACT

Non-surgical recovery and quality of eggs in spontaneously ovulating normal and repeat breeding cows revealed lower egg percent in repeat breeding recovery (28.57%) than normal (control) cows (53.33%). The quality of eggs recovered on day 7 was by and large poorer in repeat breeding than normal (control) cows. Effect of uterine flushing on subsequent estrous cycle length revealed significant decrease in the length of estrous cycle after flushing  $(23.24\pm0.52)$ 18.89±0.70 to days: P<0.01). Higher conception rate following flushing in normal cows was obtained. However, there was no improvement in conception rate in repeat breeding cow following flushing.

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Repeat Breeding is a major cause of production losses in dairy industry. The incidence of such problem has been reported 10-25% in exotic as well as Indian breeds of cattle (Rao and Kotayya, 1980; Sharma et al, 1983) Fertilization failure and early embryonic mortality have been suggested to be the major causes of reproductive failure. Occurrence of embryonic mortality in repeat breeding cows have been reported to be inconsistent, however, it was emphasised that majority of embryonic death occur around day 6 to 8 upto day 18 of the cycle (Diskin and Sreenan 1980). It has been reported that approximately 40% of the embryos are lost during first three weeks of insemination (Sreenan and Diskin, 1985). The present investigation accordingly has been undertaken to study the non-surgical recovery and quality of eggs in repeat breeding and normal spontaneously ovulating crossbred cows.

#### MATERIALS AND METHODS

A total of forty crossbred cows (Hariana crosses with Holstein Friesian, Jersey and Brown

Swiss) were selected from the Institute herd and were divided into two groups. Group A (repeat breeding cows, n=20) included those cows which failed to settle on three or more inseminations following parturition. All cows were examined (per rectum) and were not having any gross abnormalities of the genital organs. Animals were subjected to detection of estrus twice daily (6 am and 5 pm) using a vasectomized bull and visual observation of estrus syndrome. They were confirmed on estrus by rectal palpation of the genital organs. Cows were inseminated with frozen semen at 10-12 hr and 20-24 hr after the first observation of standing heat. On day 7 (day 0 = day of estrus), cows were examined (per rectum) for the presence of corpus luteum and the ipsilateral uterine horn of those cows which had palpable corpus luteum were flushed non-surgically under the gravitational method. Modified Dulbecco's phosphate buffer saline containing 0.2% bovine serum albumin fraction V was used as flushing medium. Two-way Foley's catheter (22") and an embryo concentrator (pore size 0.45  $\mu$ ) were used for flushing of the uterine horn. The quality and stage of embryonic development was determined morphologically. The evaluation of egg was done as per Shea (1981). Eggs were graded as excellent/good, fair, poor, degenerated and unfertilized.

After flushing the onset of estrus was recorded twice daily (6 am and 5 pm) with the help of a vasectomized bull and visual observation of estrus syndrome. The length of estrus cycle prior to flushing and after flushing were calculated and compared. The conception rate upto third insemination after flushing in flushed and non-flushed cows were calculated. The data were analysed as per Snedecor and Cochran (1967).

#### RESULTS AND DISCUSSION

The percent egg recovery was lower in repeat breeding cows (28.57%) than the normal

cows (53.33%) (Table 1). The lower egg recovery in repeat breeding cows compared to normal cows might be due to losses of ova into the abdominal cavity as a consequence of malfunction of oviduct (fimbria) at the time of ovulation or due to fragility of zona pellucida with subsequent degeneration and difficulties in collection (Graden et al. 1968; Linares et al. 1980). In the present investigation, 25% of the total eggs collected from repeat breeding cows had broken zona pellucida and a higher percentage of eggs were degenerated (75%) which might have affected adversely the egg recovery. Possibilities of unilateral or bilateral blockage of fallopian tube affecting embryo recovery also can not be ruled out. Higher incidence of blockage of fallopian tube in repeat breeding cattle has been reported by Kavani (1984) and Khanna and Sharma (1992). Studies, therefore, on Patency of fallopian tube in relation to egg recovery would be needed to explain precisely role of blockage of fallopian tube towards repeat breeding. Lower egg recovery in repeat breeding heifers (57%) and cows (32%) than heifers (72%) and normal cows (57%) have also been recorded by Linares et al: (1980) and Thibier et al. (1985) which is in agreement with the findings of the present investigations. The fertilization rate obtained in repeat breeding cows was higher (75%) than the normal cows (50%). However, the quality of eggs were by and large poorer in repeat breeding cows than normal cows indicating repeat breeding might be due to developmental abnormalities of the eggs rather than failure of fertilization. Higher incidence of degenerated eggs in repeat breeding heifers has also been recorded by Linares (1981) and Gustafsson (1985) which are in agreement with the findings of the present study. Fertilization rate, in both the groups, in the present investigations seem to be lower than reported in exotic cattle Linares et al. (1980a,b), Linares (1981), Gustafsson (1985). The lower fertilization rate in these cattle vis-a-vis to exotic cattle might be due to the differences in breed, endocrine profiles and agroclimatic conditions. In the present investigation one unfertilized egg surrounded by cumulus granulosa cell complex was recovered upon uterine flushing on day 7 which might be due to delayed ovulation affecting the fertilization rate. Delayed ovulation as a cause of failure of fertilization has also been reported in cattle (Boistedt, 1976). The higher incidence of egg abnormalities in repeat breeding cows might be due to the disturbances in the endocrine profiles during or after estrus. Higher

progesterone and lower levels of luteinizing hormone at estrus has been reported affecting adversely the normal embryonic development (Kesner et al. 1982; Greve et al. 1983; Moore, 1984): Endocrine situation during and after estrus has been reported more unstable in repeat breeding than virgin heifers influencing the fertilization rate and normal embryonic development (Gustafsson, 1985). Possibility of uterine infections leading to the degeneration of eggs also can not be ruled out. More investigation on this line, therefore, is needed in order to give proper explanation of poor egg quality in repeat breeding cattle.

The mean length of estrous cycle post flushing was shorter (18.89±0.70 days) than the previous cycle (23.24±0.52 days) (Table 2). The shortening of estrous cycle might be due to early release of uterine luteolytic factor induced by the pressure of the balloon and the effect of flushing on the uterine endometrium. Reduction in the length of estrous cycle by 3 to 5 days was also recorded when cows were flushed with Lugol's iodine during luteal phase of the cycle and this reduction in the length of estrous cycle was thought due to early release of PGF<sub>2</sub> alpha (Kindahl et al. 1977). Shorter estrous cycle length after non-surgical collection of eggs have also been recorded by Elsden et al. (1976) and Linares et al. (1980b).

The conception rate after flushing was higher (92.85%) in normal cows than the non-flushed normal cows (80.00%) indicating beneficial effect of uterine flushing on the establishment of conception. However, such an effect of uterine flushing on conception rate was not observed in repeat breeding cows. It appears, therefore, that factors other than uterine environment might be responsible for the repeat breeding condition. The improvement in conception rate after flushing of uterine horn might be due to the effect of antibiotics of the flushing media and washing of the uterus making the uterine environment favourable for the early embryonic development. Improvement in conception rate after flushing and collection of embryos in supervulated cows has been recorded by Haupt (1979). However, Rommel et al. (1986) have failed to demonstrate improvement in conception rate due to flushing of uterine horn.

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Table 1. Recovery rate and quality of eggs in repeat breeding and normal (control) cows

Parameters	Repeat breeding cow	Normal Cow	Overall	
Number of cows	20	20	40	
Cows having CL	15	15	30	
Cows flushed	14	15	29	
Total eggs recovered	4	8	12	
Percent egg recovery	28.57	53.33	41.38	
Fertilized eggs	3	4	7	
	(75.00)	(50.00)	(58.34)	
Unfertilized eggs	1	4	5	
	(25.00)	(50.00)	(41.66)	
Quality of fertilized eggs				
i) Good	0	0	0	
li) Fair	0	3	3	
		(37.50)	(25.00)	
iii) Poor	0	0	0	
iv) Degenerated	(75.00)	(12.50)	(33.34)	

Figures in parentheses indicate percentage

# Table 2. Effect of flushing on subsequent conception rate

Group	No.of cows	Cows Inseminated	Cows pregnant	Conception rate	Insemination per conception
Flushed cows		and the second second			and the second
i) Repeat breeder	14	13	3	23.07	2.667
ii) Normal	15	14	13	92.85	1.643
iii) Overall	29	27	16	59.26	1.937
Non-flushed					
cows					
i) Repeat	6	4	3	75.00	3.000
breeder					
ii) Normal	5	5	4	80.00	1.500
iii) Overall	11	9	7	77.78	2.143

- Bostedt, H (1976). Delayed ovulation as a cause of sterility in the A1 of cattle. 8th Int. Congr. Anim. Reprod. Artif Insem, Krakow.
- Diskin MG and Screenan JM (1980). Fertilization and embryonic mortality rates in beaf heifers after artificial insemination. J. Reprod. Fertil. 59:463.
- Elsden, RP, Hasler JE and Seidel Jr GE (1976). Non surgical recovery of bovine eggs. Theriogenology 6:523.
- Graden AP, Olds, D, Mochow CR & Mutter LR. (1968). Causes of fertilization failure in repeat breeding cattle. J. Dairy Sci. *51*:778.
- Greve T, Henrik C & Hyttel P. (1983). Endocrine profile and egg quality in superovulated cows. Nord Vet. Med. 35:408.
- Gustafsson H, (1985). Characterisitcs of embryo from repeat breeder and vigrin heifers. Theriogenology 23:487.
- Haupt P. (1979). Factors influencing superovulation success in lactating cows and their fertility after egg collection. Ph.D. thesis, FRG, 1979. (c.f. Anim. Breed Abstr, *49*:5147).
- Kavani FS, (1984). Gynaecological, microbiological and histological investigations with therapeutical considerations in repeat brreder bovines. Ph.D. thesis, G.A.U., Anand.
- Kenser JS, Padmanava V and Convey EM, (1982). Estradiol induced and progesteron inhibits the pre ovulatory surge of luteinizing hormone and follicle stimulating hormones in heifers. Biol. Reprod. 26:571.
- Khanna AK & Sharma NC (1992). Ovulatory disturbances in bovine. Indian J. Anim. Reprod. 13:43.
- Kindahl H, Granstrom E, Edquist LF, Gustafsson B, Astrom G & Stabenfeldt G. (1977). Progesterone and 15-keto-13, 14 Dihydroprostaglandin F<sub>2</sub> alpha levels in perpherol circulation after interauterine iodine infusions in cow. Acta Vet Scan. 18:274.
- Linares T. (1981). Embryonic development in repeat breeder and virgin heifers seven days after insemination. Anim. Reprod. Sci. 4:189.
- Linares, T. King WA & Polen L. (1980a). Observation on the early development of embryos from repeat breeder heifers. Nord. Vety. Med. *32*:433.
- Linares T. King, W.A., Larsson K, Gustavsson I & Bane A. (1980b). Successful repeated non-surgical collection of blastocyst from virgin and repeat breeder heifers. Vet. Res. Commun. 4:113.
- Moore RM, Kruip TAM & Green D. (1984). Intraovarian control of folliculogensis to superovulation. Theriogenology 21:103.
- Rao AVN & Kotayya K. (1980). Incidence and Causes of repeat breeding among cattle and buffaloes under field conditions in Andhra Pradesh. Indian J. Anim. Hlth. 19:121.
- Rommel P. Rehbock F. Kanitz, W & Jenichen W. (1986). Superovaluation, insemination and embryo transfer in cows. Tierzuent 40:197, (C.f. Animal Breed Astr, 55:6856).
- Sharma NC, Luktuke SN & Gupta SK. (1983). Incidence of repeat breeding in crossbred cattle. Indian J. Anim. Reprod. 3:110.
- Shea BF (1981). Evaluating the bovine embryo. Theriogenology 15:31.

Snedecor GW & Cochran WG. (1967). Statistical Method Oxford and IBH Publishing Co, Calcutta.

- Sreenan JM & Diskin MG, Martinus, Nijhoff, Dordrecht (1985). The extent and timing of embryonic mortality in the cow. In: Embryonic mortality in farm animals 1.
- Thibier M., Gouffe D., Jean O. Valognes J, Daunizeau A & Humbolt. (1985). Enhancing the rate of recovery and quality of embryos in repeat breeding cows by using a GnRH anlogue injection at mid luteal phase prior to breeding. Theriogenology 24:725.

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# Superovulation in crossbred cattle with different treatment regimens

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#### ABSTRACT

Twenty four crossbred cows were sperovulated using 36 mg of FSH-p in a tapering dose schedule (6/6, 5/5, 4/4, 3/3). The animals were divided into control (n=6) received normal superovulation protocol; regimen-1 (n=6), received 0.75 mg luprostiol, both sides at synchronized esturs; regimen-2 (n=6) received 0.75 mg luprostiol, both sides at synchronized as well as superovulatory estrus and regimen-3 (n=6) received 0.75 mg luprostiol, both sides at synchronized estrus and 8 ug of Buserelin at superovulatory estrus along with the 1st insemination. Estrus was detected twice daily and animals were inseminated 8 hrs after standing estrus and repeated twice at an interval of 12 hrs. The mean ovulation rate was 8.40±1.46, 7.50±1.19, 11.25±2.16 and 6.00±0.57 and the average number of egg recovered were 4.40±1.60, 6.50±1.19, 6.50±1.25 and 4.00±1.5 with an average number of of embryos 0.60±0.40, transferable 1.25±0.47, 2.75±0.94 and 1.33±1.33, for regimen-2 control. regimen-1, and regimen-3, respectively. Though an increase in ovulation rate and number of transferable embryo was observed in regimen-2, yet the values did not differ significantly (P>0.05) among the regimens.

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The inconsistency of response to superovulatory treatments continued to be a major limitation in the widespread use of embryo transfer technology in the third world countries. Regardless of hormone preperation and treatment regimens utilized, embryo yield and quality remains unpredictable with a large variation in developmental states, reflecting a difference in recruitment and/or ovulation times among oocytes (Shea, 1981). Literature available indicate that cloprostenol administered during late preovulatory period can enhance or synchronize ovulation in superovulated cattle (Gallo *et al.*, 1992). The present study was undertaken to see the effect of small dose of luprostiol (0.75 mg, 0.1 ml, both sides) by IVSM route at synchronized or superovulatory estrus or Buserelin (8/ug, 1/M) administered at superovulatory estrus, on superovolation.

#### MATERIALS AND METHODS

A total of 24 crossbred cows (HF, BS, Jersy) crosses with Hariana) of different parities and age (4-8 years) were superovulated using 36 mg of FSH-p (Schering Corporation, USA). The FSH was administered using four days, tapering 5/5, 4/4. 3/3. dose schedule (6/6, Superovulatory estrus was induced using luprostiol (3.75 mg, 0.5 ml), by IVSM route 48 h after initiation of gonadotrophin treatment. The animals were divided into control (n=6), receiving nomal superovulation protocol; regimen-1 (n=6), receiving 0.75 mg luprostiol both sides at synchronized estrus; regimen 2 (n=6), receiving 0.75 mg luprostiol, both sides of synchronized or superovulatory estrus and regimen-3 (n=6 receiving 0.75 mg luprostiol, both sides at synchronized estrus and 8 ug (I/M) Buserelin at superovulatory estrus along with the 1st insemination. Esrus was detected twice daily by teaser parading and visual signs of estrus. The animals were inseminated 8 h after standing estrus and repeated twice at an interval of 12 h. Number of ovulation was counted as per presence of CL palpated on day 7 per rectally. The non surgical uterine flusing was done on day 7 by gravitational method using two way Foley's cathether and the embryo quality was assessed under steriozoom Microscope (Lindner and Wright, 1983). Statistical analysis was done by Micro-32 Computer and Means were compared using 't' test (Snedecor and Cochran, 1968).

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## **RESULTS AND DISCUSSION**

Out of 24, a total of sixteen (66.66%) animals responded to superovulation treatments. The animals responded were 5/6 (83.33%), 4/6 (66.66%), 4/6 (66.66%) and 3/6 (50%) in control, regimen-1, regimen-2 and regimen-3, respectively (Table 1).

The superovulatory response was found to be highest in regimen-2 (11.25±2.17) followed by control (8.46±1.46), regimen-1 (7.50±1.19) and regimen-3 (6.00±0.57) though the difference was statistically non significant (P>0.05). The overall egg recovery was 63.70% in this study which was in accordance with the values (50-80%) reported in literature (Greve et al., 1986). The average number of egg recovered per donor were higher in regimen-1 and regimen-2 (6.50±1.19 and 6.50±1.25, respectively) followed by control (4.40±1.60) and regimen-3 (4.00±1.52). The number of transferable embryos per donor were highest in regimen-2 (2.75±0.94) followed by regimen-3 (1.33±1.33), regimen-1 (1.25±0.47) and control (U.6U±U.24) (Table 1 and Fig-1). However, these values did not differ significantly (P>0.05) among regimens. To the best of our knowledge no comparable reports are available in literature using similar protocols. A higher ovulation rate in regimen-2 may be due to a possible role of PGF<sup>2</sup>-alpha in ovulatory process which supports the findings of Gallo et al., (1992) who using a different protocol obtained a significant rise in ovulation rate of superovulated cattle following administration of cloprosterol at superovulatory estrus. The results in the present study were statistically nonsignificant which may be due to less number of respondents/group thereby increasing the coefficient of variation to a great extent. It is therefore suggested that the protocols may be investigated further with a sizeable data.

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Fig 1 Effect of different superovulatory treatments on embryo quality (in %).

92

# Table 1. Effect of different superovulatory treatments on ovulation rate, recovery and quality of embryos:

	Superovulation treatments				
Parameters studied		Control	Regimen-1	Regimen-2	Regimen-3
Superovulation response					
Animals treated		6	6	6	6
Animal responding to					
superovulation (3 CL)		5(83.33%)	4(66.66%)	4 (66.66%)	3(50%)
Animals exhibiting esrus		6(100%)	6(100%)	6(100%)	6(100%)
Ovulation rate		8.40±1.46	7.50±1.19	11.25±2.17	6.00±57
Embryo produduction					
Donors flushed		5	4	4	3
Total no.of ovulation		42	30	45	18
Donors yielding egs		4(80%)	4(100%)	4(100%)	3 (100%)
Total embryo + ova					
recovered (E)		22	26	26	12
Percentembryo recovery (%	E)	52.38%	86.66%	57.77%	66.66%
Average no.of embryo					
+ ova recovered		4.40±1.60	6.50±1.19	6.50±1.25	4.00±1.52
Embryo quality	15 M 1 M				
Unfertilized embryos (%)		2.00±0.89	4.25 1.37	2.00±1.08	1.67±0.33
and the second do a		(45.45)	(65.39)	(30.77)	(41.67)
Fertilized embryos (%)		2.40±1.24	2.25±0.25	4.50±0.64	2.33±0.98
		(54.55)	(34.61)	(69.23)	(58.33)
Degenerated embryos (%)		1.80±1.20	1.00±0.40	1.75±1.43	1.00±1.00
and the second second		(40.91)	(15.38)	(26.93)	(25.00)
Transferable embryos (%)		0.60±0.24	1.25±0.47	2.75±0.94	1.33±1.33
		(13.64)	(19.23)	(2.30)	(33.33)

Mean values bearing no superscript do not differ significantly (P>0.05)

#### REFERENCES

- Gallo, G.F., Algire, J.E., Srikandakumar, A. and Downey, B.R. (1992). Effect of a Prostaglandin F<sup>E2</sup>-alpha analogue on ovulatory response of superovulated heifers. Ani. Reprod. Sci. 27:83-90.
- Greve, T., Callesan, H. and Hyttle, P. (1983). Endocrine profiles and egg quality in the superovulated cow. Nord. Vet. Med. 23:108-121.
- Lindner, G.M. and Wright, R.W. Jr. (1983). Bovine embryos, morphology and evaluation. Theriogenology 20:407-416.
- Lindsell, C.E., Murphy, B.D. and Mapletoft, R.J. (1986). Superovulation and endocrine responses in heifers treated with FSH-p at different stages of estrous cycle. Theriogenology 26:209-219.

Shea, B.F. (1981). Evaluating the bovine embryo. Theriogenology 15:31-35.

Snedecor, G.W. and cochran, W.G. (1968). Statistical Methods. 6th ed. (Ind.), Oxford and IBH Publishing co., 66, New Delhi, India.

93

# Progesterone Supplementation and Pregnancy Rate in Recipient Crossbred Cattle

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## ABSTRACT

Forty (Taur-indicus) crossbred recipients were randomly allotted into treatment group (Gr.I) and control group (Gr.II). The Gr.I recipients (n=20) were given intramuscular injection of 500 mg 17-alpha Hvdroxy progesterone caproate. immediately after nonsurgical transfer of freshly collected embryos. The recipients under Gr.II were injected with normal saline, just after embryo transfer. The pregnancy was confirmed by rectal palpation after 60 days of transfer. The pregnancy rates in recipient cows and heifers treated with P4 were 46 and 56% compared to 14.29% and 17% in control cows and heifers respectively. The difference was significant (P<0.05) indicating that supplementation with P4 just on the day of transfer might improve pregnancy rate in recipients.

Endogenous progesterone (P4) insufficiency on the day of embryo transfer might be one of the reasons for low pregnancy rate in recipient cattle (Britt and Holt, 1988) Progesterone analogues are administered to sustain early pregnancy (Johnson *et al.*, 1958 and Sreenan and Diskin, 1983). Since P4 is responsible for maintaining a quiesient and hospitable uterus, it is logical to consider that supplementation with this hormone may improve pregnancy rates in recipients. A preliminary trial was conducted to study the effect of progesterone injection soon after embryo transfer on pregnancy rates in Taur-indicus (crossbred) cattle.

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#### MATERIALS AND METHODS

Forty crossbred (Holstein x zebu; 3:1) recipient heifers (n:15, aged between 26 to 28

months) and cows (n:25, between 1st to 4th lactation), were randomly alloted to treatment (Gr. I; n:20) and control (Gr.II; n:20) groups. All the animals were under normal farm management and had healthy reproductive tract with regular estrous cycle. The recipients were synchronized with prostaglandin analogue (Prosolvin-15mg, intervet Intrnational B.v., Boxmeer, Holland), so that their estrus would coincide with that of donors. The animals were closely obsrved for estrus daily (0600, 1400 & 1800 h).

Embryos were obtained from crossbred (HF x Zebu 3:1) donors, superovulated either with FSH-P (Folltropin, Vetrepharm, Inc., Canada) on 12th day of the cycle or PMSG (Folligon, Intervet International B.V., Boxmeer, Holland) followed with an I/V injection of monoclonal anti PMSG (Neutra PMSG, Intervet International B.V., Boxmeer, Holland), just before the first Al (Balakrishnan et al., 1993). On day 7, after first Al., the embryos were recovered nonsurgically and were assessed or graded under the stereomicroscope. The recipients were examined rectally, before transfer and the CL were assessed as excellent, good and fair or poor. Under epidural anaesthesia, fresh embryos were transfered nonsurgically into the uterine by well trained and experienced horn Vaterinarians, ipsilateral to the CL, taking all aseptic precautions. Immediatly after successful tranfer, the Gr.I recipients were administered (I/M) with a single dose (500 mg) of 17-alpha Hydroxy progesterone caproate (Duraprogen, Unichem, Bombay, India.). Two months later the recipients were palpated rectally for pregnancy.

A general linear model analysis was used to evaluate the data statistically (Steel and Torie, 1960). The model consisted of pregnancy = class of recipients + treatment interraction + embryo score x treatment interraction - error + treatment interraction.

#### RESULTS AND DISCUSSION

Duraprogen is a depot P4 preparation used in the reproductive management of farm animals. The treatment group had significantly (P<0.05) higher percentage of (50%) pregnancy (Table 1) compared to control group (Gr. II) which were in agreement with earlier reports (Wright, 1981 and Salgado and Doralden, 1984). The responses in recipient heifers were significantly higher (55.6%) than cows (45.5%). Heifers might be more suitable as recipients, because of their better body condition and virgin uterus which be more conducive for embryo might implantation. The significant improvement in pregnancy rates from 17 to 56% in heifers, and 14.29 to 45.5% in cows could be attributed to P4 supplementation Walton et al., 1986).

The mean peripheral blood P4 concentration during estrous cycle (between 4-12 days) was reported to be low (2 to 3 ng/ml) in zebu and their crosses (Agarwal *et al.*, 1976, Oyedipe *et al.*, 1986, Balakrishnan *et al.*, 1986 and Balakrishnan, 1990), compared to 4 to 10 ng/ml in B.taurus cattle (Rajamahendran *et al.*, 1976 and Gustafsson *et al.*, 1986). In such cases the recipients could be supplemented with P4 injections to improve the embryo implantation.

Significantly higher (P<0.05) conception rates in Gr. I recipients, transfered with blastocyst, expanded blastocyst or compact morula of excellent/good quality embryos (Table 2) Supplementation of P4 might have improved the uterine environment for the embryo survival and normal development after transfer. Insufficiency of P4 could be implicated as a cause for abnormal development of embryos and early embryonic mortality.

Significantly higher (P<0.05) percentages of pregnancy were recorded (43 & 20%. respectively) in Gr. I having excellent and good CL compared to Gr.II recipients (8.3 and 12.5%, respectively, Table 3). The absence of viable embryos in recipients during the first seven days (before transfer), would have resulted in low P4 secretion, compared to in-vivo fertilization. There were reports that early bovine blastocyst could be able to stimulate P4 synthesis by 6-10 days of pregnancy and thereby increasing the P4 compared concentrations significantly, to non-pregnant cows (Britt and Holt, 1988; Balakrishnan, 1990 and Batra et al., 1980). It is possible that the endogenous concentrations of P4 in recipient cattle, before embryo transfer, might not be sufficient enough to sustain the pregnancy (Remson et al., 1982 and Robinson et al., 1989). So P4 supplementation to the recipients just before or after embryo transfer would be more beneficial (Wiltbank et al., 1956).

The success of embryo transfer technology depends on the higher rates of pregnancy achieved in recipient cattle. The present results suggest that P4 supplementation following embryo transfer is beneficial under all circumstances. In view of the low P4 profiles in crossbred cattle it would be more beneficial to include P4 injection, as a routine in embro transfer programme.

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Table 1: Pregnancy rates associated with progesterone supplementation in relation to class of animals.

Class of	TREAT	MENT GROUP
Animals	Control*	Progesterone treated*
Heifers	1/6 (16.7%)	5/9 (55.6%)
Cows	2/14 (14.29%)	5/11 (45.5%)
OVERALL	3/20 (15.0%)	10/20 (50.0%)

\* Number Pregnant/Number transfered.

Means within column with different superscript differ significantly (P<0.05).

Table 2: Pregnancy rates associated with progesterone supplementation in relation to embryonic factors.

Embruaria Eastara	TREATMENT GROUP						
Empryonic Factors	Contro	jl*	Proges	terone trailer			
STAGE OF EMBRYOS							
Blastocyst	1/4	(25%)	5/9	(55.6%)			
Expanded blastocyst	1/3	(33.3%)	3/4	(75.0%)			
Morula	0/4	(0%)	0/1	(0.0%)			
Compact morula	1/9	(11%)	2/6	(33.3%)			
OVERALL	3/20	(15%)	10/20	(50.0%)			
QUALITY OF EMBRYOS	-						
Excellent	2/4	(50%)	3/5	(60.0%)			
Good	1/15	(6.7%)	7/15	(46.7%)			
Fair	0/1	(0.0%)	Nil	-			
OVERALL	3/20	(15.0%)	10/20	(50.0%)			

\* No. Pregnant/No. transfered.

Means within column with different superscript differs significantly (P<0.05.).

Table 3: Pregnancy rates associated with progesterone supplementation in relation to quality of corpus luteum in the recipients on the day of embryo transfer.

	Quality of Corpus luteum Excellent	TREATMENT GROUP							
		Control	•	Progesterone transied"					
		2/12	(16.66%)	6/14	(43%)				
	Good	1/8	(12.5%)	3/15	(20%)				
	Fair	0/0	(0%)	1/1	(100%)				
	OVERALL	3/20	(15%)	10/20	(50%)				

\* No. Pregnant/No. transfered.

Means within column with different superscript differs significantly (P<0.05).

#### REFERENCES

- Agarwal, S.P., Rahman, S.A., Laumas, V.K., Agarwal, V.K. and Ahmed, A. (1976). Studies on steroid hormones: progesterone concentration in the blood serum of zebu cows during oestrous cycle. Indian J.Anim.Sci. 47:715-719.
- Balakrishnan, M., Chinnaiya, G.P., Nair, P.G., and Jagannadha Rao. A. (1986). Studies on serum projecterone levels in zebu x Holstein heifers during pre and peripupertal periods. Anim.Reprod. Sci. 11:11-15.

Balakrishnsh; M. (1990) Interrelationship of sex chromatin and steroid hormones with concomitant reproductive efficiency in Taur-indicus (crossbred) cattle. Ph.D. thesis. Univesity of Agricultural Sciences, Barcalore.

- Balakrishnan, M., Bhaskar, B.V., Chinnaiya, G.P., Arora, V.K., Ramu, A. and Sarma, P.A. (1993). Total cholesterol concentration in relation to superovulatory responses in crossbred cattle. Therioginology 40:643-650.
- Batra, S.K., Pakwa, G.S., Suri, A.K. and Pandey, R.S. (1980). Diurnal variation of progesterone levels in milk and milk fat of crossbred cows during the oestrous cycle and early pregnancy. Anim. Prod 31:127-131.
- Britt, J.H. and Holt, L.C. (1988) Endocrinological screening of embryo donors and embryo transfer recipients: A review of research in cattle. Theriogenology 29:189-202.
- Gustafsson, H., Larsson, K., Kindahl, H. and Madej, A. (1986). Sequential endocrine changes and behaviour during oestrus and metesrus in repeat breeders and virgin heifers. Ani.Reprod. Sci. 10:261-273.
- Johnson, K.R., Ross, R.H. and Fourt, D.L. Effect of progesterone administration on reproductive efficiency. J. Anim.Sci. 17:386-390 (1958).
- Oyedipe, E.D., Voh, A.A. (Jr)., Marine, B.N. and Pathiraja, N. (1986). Plasma progesterone concentration during the oestrous cylcle and following fertile and nonfertile inseminations in zebu heifers. Br.Vet.j. 142:41-46.
- Rajamahendran, R., Lague, P.C. and Baker, R.D. (1976). Plasma progesterone levels in cycling and gonadotrophin, prostaglandin treated heifers. Can.J.Anim.Sci. 56:37-42.
- Remson, L.G., Riyssek, J.D. and Karihaloo, A.K. (1989). Pregnancy rates relating to plasma progesterone levels in recipients on the day of transfer. Theriogenology 18:365-372.
- Robinson, N.A., Leslic, K.E., and Walton, J.S. (1989). Effect of treatment with progesterone on pregnancy rate and plasma concentration of progesterione in Holstein cows. J. Dairy Sci. 72:202-207.
- Salgado, R and Doralden, L.E. (1984). The effect of intravaginal progesterone on pregnancy rates in cows receiving embryo transfers. Theriogenology 21:258.
- Steel, R.D.D. and Torrie, J.H. (1960). In: Principles and procedures of statistics with reference to the biological sciences. Pub. Mcgraw. Hill Book Company, Inc. New York.
- Sreenan, J.M. and Diskin, M.G. (1983). Early embryonic mortality in the cow: its relationship with progesterone concentration. Vet. Rec., 112:517-521.
- Walton, J.S., Martinean, N.A. and Stubbings, R.B. (1986). Pregnancy rates in Hostein embryo recipients: Effect of treatment with progesterone or clenbuterol and of natural versus induced cycles. Theriogenology 26:837-845.
- Wiltbank, J.N., Haw, H.W., Kidder, H.E., Black, W.G., Ulberg, L.C. and Casida, L.E. (1956). Effect of progesterone therapy on embryo survival in cows of lowered fertility. J. Dairy Sci. 39:456.

Wright, J.M. (1981). Nonsurgical embryo transfer in cattle embryo recipient interraction. Theriogenology 15:43-56.

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Oestrus induction and fertility in sub-oestrus crossbred cows after treatment with single and split doses of PGF 2 alpha (Dinofertin)

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## ABSTRACT

In this experiment a group of ten sub-oestrus crossbred cows received PGC  $2\alpha$  as a single dose (2 ml/10 mg) and the other group of ten cows received two split doses of (1 ml/5 mg) PGF 2 alpha (IVSM) at 24 hours interval. In split dose group all 10, (100%)cows exhibited oestrus with duration of average 75.62±2.34 hours for onset while in single dose group 9, (90%) exhibited oestrus with significantly higher (P<0.05) interval of 80.07±1.48 hours for onset of oestrus. The length of oestrus was almost similar (17.82±0.36 Vs 17.66±0.36 hours) in both groups. Treatment did not show influence on ovulatory status and the ovulation time also did not reveal significant difference. In single dose group fertility was 77.7 per cent requiring 1.29 inseminations per conception while in split dose group 1.50 it was 80 per cent requiring inseminations per conception.

Sub-oestrus in cows is a major reproductive disorder leading to a heavy economic loss to dairy industry. To induce synchronised oestrus in subestrus cows PGF 2 alpha was administered intramuscularly by Eddy (1977), Elmarini *et al.* (1983), Plunkett *et al.* (1984) and intravulvosubmucosaly (by reducing the dose of PGF 2 alpha) by Ahmad *et al.* (1985) Nenducheralathan and Kathiresan (1986) and Chatterjee *et al.* (1988).

-x---x---x---

It is observed that 25.30 per cent of cows treated with single injection of PGF 2 alpha may not show behavioural signs of heat. In these animals the progesterone decline after PGF 2 alpha injection, was temporary and followed by rebound in plasma progesterone level. Thus it appeared that single injection of PGF 2 alpha was not sufficient to lyse the corpus luteum at early stages of cycle (Cornwell *et al.* 1985).

In the animals receiving two injections of PGF 2 alpha for oestrus synchronisation with second injection reduced response would be expected due to insufficient luteolysis. Thus one potential method to increase the response to PGF 2 alpha was administration of two injections at 24 hours interval. This protocol of pulsatile delivery of PGF 2 alpha could be more effective than single injection in inducing oestrus treated during the refractory second quarter of oestrous cycle.

cycle. Thus to study obove hypothesis and to reduce the cost of treatment per animal the normal treatment of single dose by IVSM was compared with two split injections of PGF 2 alpha of half (5 mg) dose at 24 hours interval.

The present study was therefore envisage the evaluation the response of split doses of PGF 2 alpha for induction of oestrus and fertility thereafter in suboestrus crossbred cows.

#### MATERIALS AND METHODS

Twentyfour suboestrus crossbred cows (failing to show signs of oestrus but having soft well grown corpus luteum on 7-12 days of cycle) were selected for the study. The cows were sent for grazing for about 3 hours and supplimented by stall feeding with adequate quantity of fodder and concentrate mixture. First group of ten cows received a single dose of 10 mg (2 ml) of PGF 2 alpha (Dinofertin) intravulvosubmucoslly and the other group of ten received split doses of 5 mg (1 ml) PGF 2 alpha at 24 hours interval. The cows were observed for expression of oestrus by parading the teaser bull followed by rectal examination at 4 hourly interval for ascertaining time of ovulation. The conception was recorded on the non-return basis and later confirmed by per rectal examination.

#### **RESULTS AND DISCUSSION**

The injection response, the duration for onset of oestrus, length and intensity of oestrus in treatment and control group is shown in Table 1. Induction response:

The induction response in split dose group was found to be superior (100%) to that (90%) in single dose therapy. Present observations are in agreement with those of Santos *et al.* (1988), who reported superior response with split dose therapy. The overall induction response was better than that reported by Eddy (1977) Elmarimi *et al.* (1983), Chauhan *et al.* (1985). Thus it is indicated that the efficacy of split dose of PGF 2 alpha by IVSM route in suboestrus

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cows is better than single dose therapy for induction of oestrus.

## Duration for onset of oestrus:

The duration for onset of oestrus with split dose of PGF 2 alpha was  $75.62\pm2.34$  hours. It was significantly (P<0.05) less than  $80.07\pm1.48$  hours with single dose therapy (Table 1). This is also suggestive of the fact that the split dose showed a better efficacy in inducing oestrus. Santos *et al.* (1988), however reported slightly longer interval of  $79.99\pm4.50$ hours in 3 dose regimen as compared to  $73.20\pm4.90$  hours in two dose regimen. It may be pointed out that the split dose and single dose therapy was given to the experimental animals at unknown luteal stage. Thus the age of C.L. and existing progesterone level might be responsible for variation.

However, in suboestrus treated cows overall induction period was almost similar to that reported by Elmarimi *et al.* (1983) Horta *et al.* (1985) and Chatterjee *et al.* (1988). This period was less than that reported by Eddy (1977), Holy (1984), Chauhan *et al.* (1985), Nenducheralathan and Kathiresan (1986).

#### Length of oestrus:

The length of oestrus in first experimental (single dose) group was  $17.82\pm0.36$  hours which was almost similar to  $17.66\pm0.36$  hours in second (split dose) group (Table 1). In the present study the duration of oestrus in normal cyclic cows was  $18.90\pm0.49$  hours which was slighthy longer (non significant) than that of induced oestrus (Table 1).

The existing levels of ovarian and pituitary hormones in normal cyclic cows are optimum as compared to the suboestrus cows. This positive factor might have caused longer oestrus period in normal cyclic cows as compared to induced oestrus by PGF 2 alpha in suboestrus cows.

## Intensity of oestrus:

The intensity of oestrus, based on total score method in treatment and control group of animals is shown in Table 1.

The number of cows expressing intense, medium and weak oestrus, was 2(20%), 5(50%) and 2(20%) in first and 3(30%), 5(50%) and 2(20%) in second group respectively. This is indicative of the fact that the intensity of induced oestrus was almost similar in , both the experimental groups. However, in normal cyclic control group 4 (66.6%) and 2(33.3%) cows expressed intense and medium oestrus and no case was recorded in weak oestrus. Thus the intensity seemed to be better in normal cyclic than that in experimental cows. Present findings are in agreement with those reported by Singh and Kharche (1985) and Makode (1990). Ovulatory Status:

In the first group of 10 suboestrus cows, 9 expressed oestrus and 7(77.77%) ovulated at an average interval of 28.03±0.73 hours after the onset of oestrus. In second group, out of 10 cows, all (100%) expressed oestrus and 8(80%) ovulated at an average interval of 29.44±0.72 hours after onset of oestrus. There was no significant difference between two treatment groups for duration for ovulation (Table 1). In normal cyclic control group out of 6 cows, 5(83.4%) ovulated at an average interval of 31.70±0.73 hours after onset of oestrus. A significant (P<0.05) difference in the duration for ovulation between normal cyclic and treated cows was observed (Table 1). The duration for ovulation recorded in present study was less than reported by Singh et al. (1987). Rao and Venkataramiah (1990) in suboestrus cows and almost similar to that reported by Moreno et al. (1988) as far as the treatment group is concerned. The induction procedure was found to influence and reduce time for ovulation significantly as compared to normal cyclic cows. The PGF 2 alpha induced luteal regression is followed by follicular growth, maturation and ovulation when secretary pattern of LH is characterised by high frequency and low amplitude Parfet et al. (1989). Thus variation in the secretor activity of these hormones might have helped in hastening the ovulation in suboestrus cows.

## Effect of PGF 2 alpha on fertility:

Out of 9 cows induced to oestrus in first group 5(55.5%) and 2 (22.2%) conceived at induced and second subsequent oestrus respectively. The number of inseminations per conception was 1.29 with fertility rate of 77.7 per cent (Table 2). In second group out of 10 cows exhibiting oestrous 4(40%) conceived at induced oestrus and 4(40%) at second oestrus. The fertility in second group was 80 per cent requiring 1.50 inseminations per conception (Table 2). It is evident that in second group the fertility was better Santos *et al.* (1988) reported higher fertility with split doses of PGF 2 alpha and attributed it to more number of cows expressing oestrus and hastening luteolysis.

Thus split doses of PGF 2 alpha could improve oestrus induction interval, oestrus length and fertility to first two inseminations as observed in present study. These results agree earlier observations of Cornwell *et al.* (1985) suggesting that two injection PGF 2 alpha administered at 24 hours interval are more effective than a single injection in luteolysis, when administered at early stages of cycle.

Table	1:	Induction	response,	duration	for	induction,	length,	intensity,	ovulatory	status	and	ovulation	time	of	oestrus
		treatment	and contro	ol group o	of su	boestrus a	ind nom	nal cyclic	cows.						

Group	No.of animals	Induction	Duration for Induction	Length of oestrus	Intensity	Intensity Ovulatory Status			
			(hours)	(hours	I N	V	0	ANO	(hrs.)
I Experimental (Single dose) T <sub>1</sub>	10	9(90)	80.07±1.48	17.82±0.36	2(20) 5(50)	2(20)	7(77.7	7) 2(22.2)	28.03±0.73
II Experimental (Split dose) T <sub>2</sub>	10	10(100)	75.62±2.34	17.66±0.36	3(30) 5(50)	2(20)	8(80	) 2(20)	29.44±0.73
			3.27	0.32 NS					1.38 NS
III Suboestrous Control C <sub>1</sub>	4	1(25)	1-15	18.70		1(25)	1(25	) —	31.20 NS
V Normal cyclic control C <sub>2</sub>	6	6(100)		18 <u>.90±0</u> .49	4(66.6)2(33.3	) -	5(83.3	1) 1(16.17)	31.70±0.73
T' values for									
T <sub>1</sub> Vs C <sub>2</sub>				1.77NS					3.55'
$T_2$ Vs $C_2$				2.04NS					2.20'

Figures in parenthesis indicate percentage

I = Intense M = Medium W = Weak

0 - Ovulatory ANO-Anovulatory Significant at P 0.05 NS+ Non significant.

Ahmad T, N. Singh and R.D. Sharma (1985): Induction of centrus in cows using PGF 2 alpha via intravulvosubnucosal route. Ind. J. Anim. Reprod. 6(2):170-171.

- Chatterjee A, K.G. Kharche and M.S. Thakur (1988): Use of prostaglandin F 2 alpha in the treatment of subcestrus crossbred cows. Ind. J. Anim. Reprod. 10(2):185-187.
- Chauhan F.S.F.Ö.K. Mgongo B.M. Kessey and S. Gombe (1985): Effect of IVSM cloprostenel injection on hormonal profile and fertility in suboestrus Cattle. Theriogenology 26(//69-75.

Cornwell, D.G., J.F. Hentages and M.J. Fields (1985): Cited by Santos et al. (1988).

- Eddy, R.D. (1977): Cloprostenol as a treatment for no visible cestrus and cystic ovarian disease in diary cows. Vet. Rec. 10062-65.
- Elmarimi, A.A. G.D. Gibson, D. Morrow, J. Mateniuk and B. Gerloff (1983): Use of PGF 2 alpha in the treatment of unobeserved sestrus in lactating dairy cattle. Amj. Vet. Res. 44(6):1081-1084.
- Holy L. (1984): Submucusal vestibulo administration of cloprosterenol in pows in relation to cestrus activity, Conception and progesterone level in milk Veterinaria Medicine 29(9):513-519.
- Horta A.E.M., C.M.S.G. Costa, J. Ralits Silva and M.I. Riosvasques (1985): Possibility of reducing the luteolytic dose of eloprostenol in cyclic dairy cows. Theriogenology 25(2):252-301.
- Makode T.P. (1990): Synchronisation of bestrus and fertility in PGF 2 alpha (Dinoprsot) treated repeat breeder cows M.V.Sc. thesis in Animal Reproduction. P.K.V., Akola.
- Moreno I, C.S. Galina, H. Bashte, Nowarro and R. Firro (1988): Comparative study of time of ovulation following oestrus synchronised with PGF 2 alpha and non synchronised one in Indo Brasisian cows. Proceeding Int. Nat. Con. on Ani. Reprod. and A.I. Dublin 1980 No.449 Anim. Breed. Abstr. 57(1) 193.
- Parfet J.R.C.A. Smith D.L. Cook, D.M. Skyer, R.S. Young quist and H.A. Garverich (1989): Secretary Patterns of L.H. and FSH and follicular growth following administration of PGF 2 during the early luleal phase in cattle. Theriogenology 31(3):513-514.
- Plunkett, S.S; J.A. Stevenson and E.P. Call (1984): Prostaglandin F 2 alpha for lactating dairy cows with palpable corpus luteum but unobserved oestrus. J. Dairy Sci. 67(2):380-387.
- Nenducheralathan, B and D. Kathiresan (1986): Intravulavosubmucosal injection PGF 2 alpha; A method of induction of oestrus. Cherion 15(2):58-59.
- Rao, A.V.N. and P. Venkatramiah (1990): Studies on the effectiveness of a smaller dose of PGF 2 alpha in increasing reproductive efficiency of ongole cattle. Ind. Vet. J.67 (6):528-530.
- Santos, E.A., A.C. Warnick, J.R. Chenault, D.L. Wakeman and M.J. Fields (1988): A novel approach for PGF 2 alpha cestrus synchronization in beef cattle. Proc. Int. Conf. on Ani. Reprod. and A.I. Dublin 1988 No.459.

Singh, M.M and Kharch K.G. (1985): Sexual behaviour and reproductive efficiency. Livestock Advisor 10(4):9-13.

Singh, G, G.B. Singh and R.D. Sharma (1987): Ovulation and Fertility after intra vulvo submucosal injection of PGF 2 alpha in suboestrus buffaloes Ind. J. Dairy Sci. 11(2):324-325.

## A Kid Born By Transfer of Vitrified Embryos in Goat

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Cryo-preservation of embryos besides being a valuable adjunct to the embryo transfer technology is also important for conservation of valuable genetic material and those species/breeds which are in danger of extinction. The classical method of embryo preservation in most of the farm and laboratory animals involves slow cooling (1°C/min) to 30°C, to-60°C or even below before transfer to liquid nitrogen (Wlladsen, 1977: Lehn-Jensen, 1984, Rao et al., 1988; Agrawal and Polge, 1989<sup>b</sup>). The techniques used in it need expensive controlled biological freezer. Time required supervise cryo-preservation to the procesures in conventional methods is also too much. preservation of embryos by vitrification is now a new promising approach in the field of cryobiology (Rall and Fahy, 1985; Massi et al., 1987; Agrawal and polge, 1989<sup>a</sup>). In the process of vitrification by adding high concentration of cryoprotective agents, a solution is made so viscous that it solidifies without the formation of ice. There has not been any report on the success of vitrifying caprine embryos based on birth. In the present experiment, in-vivo survival of late stage embryos caprine (morula-blastocyst) vitrified by using mixture of two cryoprotectants (glycerol, 1-2 propane-diol) has been studied.



#### MATERIALS AND METHODS

The vitrification method detailed by Agrawal and polge (1989<sup>e</sup>) was used in this experiment. Embryos were recovered surgically from super-ovulated does. Morphologically normal embryos (morula-blastocyst) after 2-3 washings in Dulbecco's PBS enriched with 20% oestrous goat serum were exposed to intra cellular cryoprotecting medium (10% glycerol, 20% 1-2

propane-diol in PBS) for 10 minutes. Embryos were then transferred to extra cellular vitrification medium (25% glycerol, 25% 1-2 propane-diol in PBS). The temperature of cryoprotecting and vitrification media during embryo exposue was maintained at 4°C in an ice bucket. 2-3 embryos in vitrification medium and diluent (1 M Sucrose) in determined proportions were loaded in 0.25 ml straw. The straw was plunged in LN2 in two steps (i.e. the portion of the straw up to the level of embryos in vitrification medium plunged immediately and then rest of the portion progressively). After 1-3 months in LN<sub>2</sub> the straws were taken out and thawed in water bath maintained at 20°C. The contents of the straw were mixed by shaking, emptied in a petri dish and left for 10 min at 4°C maintained in an ice bucket. Sucrose was gradually diluted in 0.5 M and 0.25 M sucrose in PBS. After 3 washings in PHS, embryos were transferred to synchronous recipients for in-vivo development. Pregnancy in recipients was monitored by medata ultrasonic pregnancy detector.

## RESULTS AND DISCUSSION

Twenty morphologically normal embryos (morulablastocyst stage) were cryopreserved by vitrification method. One out of 20 embryos was damaged during cryo-protectant treatment before plunging in LN<sub>2</sub>. Fourteen embryos were thawed in a water bath (20°C) after 1-3 months storage in LN<sub>2</sub>. Recovery was 100 percent (14/14). Zona pellucida of three embryos out of 14 recovered was damaged during cryoprotectant/sucrose dilution. Remaining embryos exhibited too much shrinkage which ultimately returned to their initial volume after washing in 0.5M, 0.25M and 00.00M sucrose solution. Twelve embryos including one with damaged zona pellucida were transferred surgically in 5 recipients (2-3 embryos/recipient). Three recipients conveived. There was possibility of resorption of fetus in one recipient which returned to oestrus 59 days after transfer. Another recipient aborted at 90 days of transfer. A healthy

male kid of Sirohi breed weighing 4.0 Kg was born on 2nd March, 1993 by transfer of vitrified embryos to a Jakhrana doe (surrogate mother). The kid reared under routine farm management conditions is growing well and has attained a weight of 30 Kg at 12 months of age. Acknowledgement: We thank Director of the Institute for providing the facilities. The work has been done in a project entitled, "Cryo-preservation of caprine oocytes/embryos" funded by Department of Biotechnology, Ministry of Science & Technology, Govt. of India and Indian Council of Agricultural Research New Delhi.

## REFERENCES

- Agrawal, K.P. and Polge, c. 1989<sup>a</sup>. Cryo-preservation of mouse embryos at -196<sup>o</sup>C vitrification. Indian J. expt. blol. 27:383-84.
- Agrawal, K.P. and Polge, C. 1989<sup>b</sup>. Survival of mouse embryos after being frozen in glycerol-Sucrose mixture. Indian J. expt. biol 27:474-75.
- Lehn-Jensen, H. 1984. Deep freezing of cattle embryos, 10th International Congress on Animal Reproduction and Artificial Insemination (Urbana, USA) 1984, Vol-IV, II, 1-22.
- Massip, A., Van Der Zwalmen, P. and Ectors, F. 1987. Recent Progress in Cryo-preservation of cattle embryos. Theriogenology 27:69-79.
- Rall, W.F. and Fahy, G.M. 1985. Ice-freeze Cryo-preservation of mouse embryos at 196℃ by vitrification. Nature, 313:573-75.
- Rao, V.H., Sarmah, B.C., Agrawal, K.P..., Ansari, M.R. and Bhattacharyya, N.K. 1988. Survival of goat embryos frozen and thawed rapidly. Anim. Reprod. Sci. 16:261-64.
- Willadsen, S.M. 1977. In Freezing of Mammalian Embryos. edited by K. Elliot and Whelan, J. (Ciba Found. Symp. NO. 52, new series, Elasevier/North Holland, Amsterdam 1977, 175.

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# Clinico-Haematological Evaluation of two Anaesthetic Regimens for Embryo Transfer in Goats\*

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Surgical embryo transfer in goats require an ideal anaesthetic state which will facilitate maximum embryo recovery from donors and better pregnancy rate and kidding rate in recipients. Use of inhalation anaesthetics require special equipments, hence intravenous or intramuscular agents are preferred to practice the technique in field condition. In addition, the commonly used inhalation agents like nitrous oxide, and halothane respectively resulted in reproductive loss (Mazze *et al.*, 1986) and early abortion (Critchlow *et al.*, 1991).

The desirable qualities in selection of anaesthetics for embryo transfer programmes could be, an anaesthetic or combination which would anaesthetic render maximal embryo retrieval from donors and would not interfere with the embryo or life of the embryo in the recipients till kidding. In addition muscular relaxation, easy exteriorization and manipulation of uterus and associated structures are also warranted in both collection and transfer to prevent post operative adhesions. Hence the present study was conducted to evaluate two anaesthetic regimen with the commonly used agents diazepam (Singh and Kumar, 1988). Xylazine (Kumar et al., 1986) and ketamine (Coulson et al., 1991).

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## MATERIALS AND METHODS

The study was conducted on 6 donors and 12 recipients programmed and prepared for embryo collection and transfer. The does were divided equally into group I and II.

Ketamine (11mg/kg) was administered as intravenous injection 15 miniutes after intramuscular injection of xylazine in group I, Kelamine at dose rate of 15 mg/kg was given 5 minutes after intravenous injection of diazepam in group II. The parameters studied were quality and duration of induction, anaesthesia and recovery. The clinical parameters studied were respiratory rate, heart rate and rectal temperature. The haematological parameters included were haemoglobin, total erythrocyte count, total leucocyte count and packed cell volume. The parameters were studied before sedation, before induction, after induction, after handling uterus, after closure of laparotomy and after recovery. The other parameters studied were skeletal muscle relaxation, embryo retrieval rate in donors and pregnancy rate in the rcipients.

## RESULTS

The two anaesthetic regimens for embryo transfer under study provided smooth induction with the induction time 2.62±0.35 minutes in group I and 3.06±0.44 minutes in group II. The duration of anaesthesia was significantly shorter (P>0.01) in group II (20.46±1.84 minutes) as compared with group I (44.33±3.61 minutes). The quality of anaesthesia was excellent in relation to muscle relaxation and exteriorization of uterus in group I. Spontaneous movements, limb hypertonicity of muscles were noticed in group II. Group II donors received a second injection of ketamine (5.00 mg/1 kg BW) to maintain anaesthesia for completion of embryo collection. No significant differences were noticed in the recovery time (22.96±2.21 VS 17.28±2.15 minutes). The recovery was smooth. No incidence of regurgitation or aspiration was noticed in both the groups. Swallowing and pelpebral reflexes were persistant in both the groups throughout the anaesthetic period.

The mean respiratory rate in both xylazine and diazepam premedicated groups decreased

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significantly (P<0.01) after sedation. No significant changes were noticed in the heart rate and mean rectal temperature. No significant changes were noticed in both the groups with respect to haemoglobin, total erythrocytic count, total leucocytic count and packed cell volume.

The intra tubal embryo retrieval rate was 96.42 per cent in xylazine premedicated donors whereas the same was 90.61 per cent in diazepam premedicated donors. But handling of uterus and cannulation were easily performed in xylazine premedicated does. The success rate in recipients was 66.66 per cent in xylazine and zero per cent in diazepam premedicated groups.

#### DISCUSSION

The induction was quicker in both xylazine and diazepam premedicated does because of ketamine binding ability with oplate receptors (Finck and Nagai, 1982). When ketamine was combined with xylazine, the duration of anaesthesia was significantly increased due to the adjunct action of xylazine. The spontaneous movements, limb stretching and facial muscle tremors during induction and anaesthesia in diazepam premedicated does could be due to the hypersynchronous activity of ketamine on cortical area (Tamasy *et al.*, 1975). Ketamine in combination with xylazine provided excellent muscle relaxation resulting in easy handling of uterus because xylazine was a centrally acting muscle relaxant (Samy and Tantawy, 1981). The hypertonicity induced by ketamine was masked by xylazine (Amend *et al.*, 1972). The vital reflexes like deglutition, pharyngeal palpebral reflexes were intact and were protected by ketamine (Ramakrishna *et al.*, 1981).

The respiratory depression could be due to the effect of xylazine on medullary centres (Leela and Bhlokre, 1985) and of diazepam on enhancing the inhibitory neurotransmittors - r amino butyric acid (Muir and Hubbell, 1991). Though xylazine interfered with carotid sinus baroreceptors reflex response (Garner et al., 1971) and diazepam induced bradycardia (Muir and Hubbell, 1991) ketamine maintained the heart rate due to its stimulation of sympathetic nerve trunks (Thurmon et al., 1973), inhibition of vagal component of the baroreceptor reflex (McGrath et al., 1975) or selective positive inotropic influence in heart muscle (Adams et al., 1977) or increase in epinephrine level (Craft et al., 1983).

The less pregnancies in diazepam premedicated does could be due to the increased dose of ketamine (15 mg/kg BW) which could increase uterine vascular resistance and reduce the blood flow (Craft *et al.*, 1983).

#### REFERENCES

- Adams, H.R., J.L. Parker and B.P. Mathew. 1977. The influence of ketamine on ionotropic and chronotropic responsiveness of heart muscle. J. Pharmacol. Exp. Ther. 201:171-183.
- Amend, J.F., P.A. Klavano and E.c. Stone, 1972. Premedication with xylazine to eliminate muscular hypertonicity in cats during ketamine anaesthesia. VM/SAC 67:1305-1307.
- Coulson, N.M., A.J. Janusz, R. Kiewioz and G.R. Ripple, 1991. Physiological responses of sheep to two hous anaesthesia with diazepam ketamine. Vet. Rec. 129:329-332.
- Craft, J.B., L.A. Coaldrake, M.L. Yonekura, S.D. Dao, G.C. Evelyn, M.F. Roizen, P. Mazel, R. Gilman, L. Shokes and A.J. Trevor, 1983. Ketamine, catecholamines and uterine tone in pregnant ewes. Am.j. Obstet. Gynecol. 146429-433.
- Critchlow, B.M., Z. Ibrahim and B.J. Pollard, 1991. General anaesthesia for gamete intrafallopian transfer. Eur. J. Anesthesiol. 8:381-384.

Finck, A.D. and S.H. Nagai, 1982. Oplate receptor mediation of ketamine analgesia. Anesthesiol 56291-297.

Garner, H.E., J.F. Amend and J.P. Rosborough, 1971. Effects of Bay VA. 1470 on Cardiovascular parameters in ponies. VM/SAC., 66:1016-1021.

Kumar, A., S.K. Pandey, V.P. Chandrapuria and S.M. Shrivastava, 1986. A note on clinical evaluation of different ketamine combinations in goats. Indian J. Anim. Sci. 18:39-43.

Leela, C.M. and A.P. Bhokre, 1985. Evaluation of xylazine as an anaesthetic agent in combination with certain preanaesthetic drugs in dogs. I Respiratory rate, blood pressure, heart rate and temperature. Indian Vet. J. 42:1125.

- Mazze, R.I., M. Fujinaga, S.A. rice, S.B. Harris and J.M. Baden, 1986. Reproductive and teratogenic effects of nitrous oxide, halothane, isoflurane and enflurane in spragne Dawley Rats. Anesthesiol 64:339-344.
- McGrath, J.C., J.E. Machenizie and R.A. Millar, 1975. Effects of ketamine on central sympathetic discharge and the baroreceptor reflex during mechanical ventilation. Br. j. Anesth. 47:1147-1149.

Muir, W.W. and J.A.E. Hubbell, 1991. Handbook of Veterinary Anesthesia. I Edn. Jaypee Brothers, New Delhi.

- Ramakrishna, O., D. Krishnamurthy and J.M. Nigam, 1981. Ketamine anaesthesia in buffalo calves. Indian Vet. J. 58:503-505.
- Samy, M.T. and M. Tantawy, 1981. The clinical application of combined Rompun and MY 301 in buffaloes. Vet. Med. Rev. 85:72-79.
- Singh, B. and A. Kumar, 1988. Diazepam as preanaesthetic to thiopentone anaesthesia in goats. Indian Vet. J. 65:314-319.
- Tamasy, V., L. Koranyi and M. Tekeres, 1975. E.E.G. and Multiple unit activity during ketamine and barbiturate anaesthesia. Br.J. Anesth. 47:1247-1250.
- Thurman, C., A. Kumar and R.P. Link, 1973. Evaluation of ketamine hydrochloride as an anaesthetic in sheep. J.A.V.M.A. *162*:293-297.

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## Effect of hormone induced estrus in Madras Red ewes during summer

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## ABSTRACT

Forty parous ewes were subjected randomly to three treatment regimes for induction of estrus and fertility during summer (May — June). In progesterone treated group 60 percent of ewes showed estrus, 40 percent lambing percentage achieved. In PGF2x and GnRH group 30 percent and 10 percent of ewes showed estrus respectively and lambing percentage were 10 percent for each group. The interval of onset of estrus from treatment and estrus duration were 96h, 60h, and 216h and 18h, 15h and 18h respectively for progesterone PGF2x and GnRH treated group.

The ability of a single injection of GnRH to induce a pre-ovulatory type LH and FSH surge release and ovulation in anoestrus cows (Zolman *et al.*, 1974) has led to its wider application in farm animals. However, the results are variable and the induced cycles are often of shorter duration due to difficient luteal function (Kesler *et al.* 1978).

-x---x-

Prostaglandin F2 alpha has been reported to cause functional and structural luteolysis in sheep (Uaro, 1975). Estrous synchronization in ewes with progesterone administration through systamic route as well as Vaginal passaries have been reported by various workers (Davies 1960).

The present study was undertaken to assess the efficacy of GnRH, PGF<sup>2</sup> x and progesterone treatment in ewes to augment fertility during summer.

#### MATERIALS AND METHODS

Forty parous ewes were divided equally at random into four groups and subjected to

respective treatment for induction of estrus in summer (May-June). Group 1:- progesterone (Duroprogen) injection 15mg i.m. for 10 days and PMSG 400 I.U. i.m. at last progesterone injection. Group 2:- Two PGF<sup>2</sup>x (lutalyse) injection i.m. 5mg at 10 days interval. Group 3:- GnRH single injection (Receptal) 2.5ml i.m. Group 4:- No treatment as control will be left in other three treated groups. After withdrawal of treatment the animals were detected for estrus at 12hr. interval by using an aproned buck and hand-mating was followed.

### **RESULTS AND DISCUSSION**

Goel and Agrawal (1991) reported 66.66% of does exhibited estrus with vermix vagimal sponge (60 mg Medroxy Progesterone acetate, Upjohn) during summer. He has also concluded that prostaglandin analogues are not effective in inducing estrus during summer season which could be due to absence of corpus luteum development during summer period. Our data are also agree with his findings. Poor response to GnRH could be due to lack of sensitivity of gonadoptropin producing pituitary cells. Narasimha Rao (1991) has observed that E<sup>2</sup> pre-treatment augment the pituitary responsiveness to GnRH for LH release compared to single GnRH in anoestrus cows. On the basis of findings in the present experiment, it is observed that progesterone treated group responded better than single GnRH injection and prostagland in analogues groups during summer. a trend of seasonal anoestrus (acyclicity) observed in Madras Red ewes.

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## Table 1.

SI.	Oestrue, conception and lambing	Treatment group							
No.	Behaviour	Progesterone	PGF2x	GnRH	Control				
1.	No. of ewes	10	10	10	10				
2.	% of ewes in estrus	60	30	10	-				
3.	Interval between treatment and onset of estrus	96	60	216	-				
	(hrs)								
4.	estrus duration	18	15	18	-				
5.	% of ewes conceived	40	10	10	-				
6.	Lambing percentage	40	10	10	-				

#### REFERENCES

- Davics, H.L. (1960). Reduced fertility associated with the use of multiple injection of progesterone by PMSG Aust. Vet. J. 36:20.
- Goel, A.K. and Agarwal, K.P. (1991). Induction and Synchronization of oestrus in Anoestrous (Acyclic) Recipient - goat. IJAR 12:187-189.
- Kesler, D.J., Gavverick, H.A., Young quist, R.S. Elmore, R.G. and Bierschwal, C.J. (1978). Overian and endocrine responses and reproductive performances following GnRH treatment in early post-partum Cows. Theriogenology 9:363
- Narashimha Rao, A.V. (1991) Interaction of Gonadotroptin releasing hormone and oestradiol on pituitary and ovarian responsiveness in Anoestrus cows. IJAR 12:155-158.
- Umo, T (1965) Effect of PGF<sup>2</sup>x on the ultrastructure and function of sheep corpura lutea. J. Reprod. Fert. 43:287-292

Zolman, J., Convey, E.M., Britt, J.H. and Hafs, H.D. (1974).

Relation ship between the luteinizing hormone responses to gonadotropin releasing hormone and endogenous steroids. J. Anim. Sci. 39:355.

# Circulatory levels of Thyroid Hormones during different stages of reproduction in Goat

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## ABSTRACT

The serum triiodothyronine  $(T_3)$  and thyroxine (T<sub>4</sub>) concentrations of 75 different indigenous goats of Assam were estimated by RIA. These animals were at different stages of reproduction i.e. on the day of oestrus, early pregnancy (0-50 days), mid pregnancy (50-100 days), late pregnancy (100 - 145)days) and non-pregnant (control). The average concentration of T<sub>3</sub> and T<sub>4</sub> on the day of oestrus, early pregnancy, mid pregnancy, late pregnancy and non-pregnant were found to be 1.68  $\pm$  0.11, 1.42  $\pm$  0.08, 1.48  $\pm$  0.12, 1.54 ± 0.10 and 0.78 ± 0.10 ng/ml and 88.10  $\pm 2.91,70.40 \pm 3.24,75.50 \pm 4.64,80.24$ 2.61 and 52.42 ± 3.21 ng/ml respectively. The mean levels of T<sub>3</sub> and T<sub>4</sub> showed the peak value on the day of oestrus and similarly the lowest value in non pregnant condition respectively.

Recently, emphasis has been given to establish a correlation between the endocrine function of the body and reproductive efficiency of female animals. The hormonal profile of thyroid gland varies during different reproductive stages (Khurana and Madan, 1986 and Baruah et al., 1993). In humans, hypothyroidism is believed by many to be closely associated with sterility, infertility and abortion. Similarly in cattle, thyroprotein or triiodothyronine may be of value in certain type of female infertility such as "silent" oestrus (Roberts, 1971) and thyroid activity is necessary for normal reproductive function. Moreover, during the period of gestation a sequence of changes occur in the physiology of animals including a change in body metabolic rate which is mainly controlled by thyroidal

x----x-----x-----

hormones (Yousef and Johnson, 1975). Although many scientists have worked on thyroidal activity in different re-productive status, no information is available about the thyroidal hormones in different reproductive status in indigenous goat (carprahircus L) of Assam. The present investigation was therefore conducted.

## MATERIAL AND METHODS

Experimental animals: Observations were made on groups of fifteen female indigenous goats of Assam (between 2-3 years of age) in oestrus, early pregnancy (0-50 days), mid pregnancy (50-100 days) late pregnancy (100-145 days) and non-pregnant (control) conditions.

**Nutrition and Management:** During the experimental period, the ambient temperature and relative humidity (RH) were 26.2°C and 82.3% (Autumn season). The animals were maintained under semi-intensive system of rearing. They were allowed to graze during morning hours and in the evening 300 gm of concentrate mixture, fed to each animal. Water was available ad-lib.

**RTA** technique: Blood samples were collected from each of the experimental animals of the different groups i.e. in oestrus, early pregnancy, mid pregnancy, late pregnancy, and non-pregnant. The serum was separated from blood samples and stored at  $-20^{\circ}$ C till the assay was carried out. All serum samples were assayed for T<sub>3</sub> and T<sub>4</sub> using RIA kit supplied by BARC, Bombay as per protocol supplied with the kit. All samples were assayed in duplicate and were critically evaluated.

Statistical analysis: The data were analysed statistically as per the standard methods

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given by Snedecor and Cochran (1967). Critical difference (C.D.) test was done to see the significant difference of T<sub>3</sub> and T<sub>4</sub> values in different reproductive conditions.

#### **RESULTS AND DISCUSSION**

The different levels of trijodothyronine  $(T_3)$ and thyroxine (T<sub>4</sub>) during different reproductive conditions in indigenous goats of Assam are presented in table 1. The T<sub>3</sub> and T<sub>4</sub> values were pronounced in oestrus and pregnancy states in the sequence given. The T<sub>3</sub> and T<sub>4</sub> concentrations in the blood of goat varied significantly (p>0.01) between different stages of reproduction. As revealed in the C.D. test (at 5% level), concentrations of T<sub>3</sub> and T<sub>4</sub> were found to be higher on the day of oestrus than the non-pregnant condition. The intra and inter assay coefficients of variation for T<sub>3</sub> and T<sub>4</sub> assays were assays were 5.2% and 9.8% and 5.4% and 11.6% respectively.

The apparent increase in T<sub>3</sub> and T<sub>4</sub> levels, towards late pregnancy from the day of oestrus were guite similar with the observations on thyroid activity made by Sharma and Sharma (1976) in goat and Hassan and El-nouty (1985) and Kan and Crueses (1987) in bovine.

during different reproductive stages.

The higher levels of T<sub>3</sub> and T<sub>4</sub> during oestrus may possibly be associated with the increase of oestrogenic activity of the body. Concentrations of T<sub>3</sub> and T<sub>4</sub> appeared to be closely associated with the increase in concentraions of circulating oestrogen (Ingbar and Woebar, 1974). Williams (1974) also reported that administration of oestrogen or androgen caused alteration in the binding of thyroid hormones in plasma and elevation of T<sub>3</sub> and T<sub>4</sub> concentration in blood might be due to the increased concentration of TBG as a result of high oestrogen level. Similarly, the observed rise in serum T<sub>3</sub> and T<sub>4</sub> particularly during late pregnancy may be attributed to the changes in gonadal hormone secretion. In the late pregnancy oestrogen secretion has been observed to increase markedly (Rao et al., 1978). High level of oestrogen increase the secretion of thyrotropin and thyroxine which participate in stimulating milk secretion after parturition. Moreover, increase in T<sub>3</sub> and T<sub>4</sub> levels towards late pregnancy from the oestrus might be due to increase BMR during later half of pregnancy owing to increase metablic demand of the foetus (Searcy, 1969).

Table 1. Serum Triiodothyronine  $(T_3)$  (ng/ml) and Thyroine  $(T_4)$  (ng/ml) levels in goats

Reproductive stages	T <sub>3</sub>	T <sub>4</sub>
Oestrus	1.68 <sup>a</sup> ± 0.11	88.10 <sup>a</sup> ± 2.91
Early Pregnancy	1.42 <sup>a</sup> ± 0.08	$70.40^{a} \pm 3.24$
Mid pregnancy	1.48 <sup>a</sup> ± 0.12	<b>75</b> .50 <sup>a</sup> ± 4.64
Late Pregnancy	1.54 <sup>a</sup> ± 0.10	80.24 <sup>ª</sup> ± 2.61
Non-pregnant (control)	0.78 <sup>b</sup> ± 0.10	52.42 <sup>b</sup> ± 3.21

REFERENCES

Baruah, K.K.; Baruah, Anubha and Baruah, R.N. (1993). Circulatory levels of thyroid hormones during oestrous cycle in dairy cows. Ind. J. Anim. Reprod. 14(2): 72-73.

Hassan, G.A. and El-nouty, F.D. (1985). Thyroidal activity in relation to reproductive status, seasonal variations and milk production in buffalo and cow heifers during their first lactation. Indian J. Dairy Sci. 38(2) : 129-135.

Ingbar, H.S. and Woebar, A.K. (1974). The thyroid gland (pp 124, CF Text Book of Endocrinology, Edited by William, H. Roberts, W.B. Saunders Company, Philadelphia).

Kan, K.W. and Cruess, R.L. (1987). Gestational changes of thyroid hormones action in the developing foetal bovine epiphysis. Calcified Tissue International. 41(6): 332-336 (C.F. Vet. Bull. 1988, 58(5): 3066).

- Khurana, M.L. and Madan, M. (1986). Effect of stage of pregnancy on circulating thyroidal hormones among Karan swiss and Murrah animals. Indian J. Dairy Sci. 39(2): 128-132.
- Rao, B.R.; Dugwekar, Y.G.; Bapat, S.T.; Gujrathi, V.G. and Varman, P.N. (1978). "Total estrogen and progesterone levels in blood of the buffaloes during the last two months of pregnancy". Indian J. Dairy Sci. 31: 181.
- Poberts, J. Stephen (1971). Physiology of female reproduction. In Veterinary Obstetrics and Genital Diseases. PP. 351. CBS Publishers and Distributions, New Delhi, India.

Searcy, R.N. (1969). "Diagnostic Biochemistry". McGraw Hill Book Company, New York.

Sharma, D.P. and Sharma, Anitha (1976). Protein bound iodine levels during oestrus, pregnancy and non-pregnancy states in goats. Indian J. Physiol. Pharmac. 20(1): 242-244.

Snedecor, G.W. and Cochran, W.G. (1967). Statistical Methods, 6th ed. The Iowa State Univ, Press, Ames.

Williams, H. Roberts (1974). In Text Book of Endocrinology. 5th ed. P. 195. Saunders Company, Philadelphia.

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Yousef, M.K. and Johnson, H.D. (1975). Am. J. Physiol. 229: 427.

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# Transitory Changes of Hormones in Serum of Postpartum Dairy Cows

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## ABSTRACT

Thirtynine dairy cows were assigned to 13 groups according to the days following parturition. Blood samples were collected on days, 1,3,6,7,8,9,10,12,14,19,21,26 and 28 postpartum at 2 hrs intervals for a 24 hours periods. Serum LH, FSH, estradiol-17B and progesterone were measured. Serum LH and estradiol-17B tended to increase with days postpartum, however, serum FSH and progesterone did not change during postpartum. Serum LH and estradoil-17B concentration were significantly correlated from day 1 to day 28 of the postpartum.



A detailed knowledge of the pattern of gonadotrophins release is important in understanding the factors controlling the return of ovarian activity in the postpartum cow. Although the hormonal profiles during the postpartum period in dairy cows were reported by various workers but studies on frequent sampling during gonadotrophin profile period for this determination seemed to be lacking. The results of such as investigations is reported in this paper

#### MATERIALS AND METHODS

Thirtynine dairy cows were assigned randomly at calving to 13 postpartum groups. The cows were subjected to the general management system applied on the farm and were fed hay, concentrate feed mixture and green fodder, whenever available, according to their maintenance requirements for and milk production. The experimental animals were at parturition after i.e., days different 1,3,6,7,8,9,10,12,14,19,21,26 and 28 days. One day before the begining of the experiment, an indwelling catheter was placed in each jugular vein of every animal and blood samples were collected at 2 hrs interval for 24 hrs. Serum was separated from blood and stored at -20°C until analyzed for hormone.

homologus, double antibody A radioimmunoassay was used to measure serum LH and FSH concentration. LH was determined following Stupnicki and Madej (1976). FSH was determined as per method of Stankov (1983). (NIAMDD-OFSH-1-1) Ovine FSH was radioiodinated and purified on Ultrogel. ACA-G4 (LKB, Sweden) to give maximum purity of the tracer and increase specificity of the assay (Stankov et al. 1986). Serum progesterone and estradiol-17B were measured according to the methods of Kanchev et al. (1976) and Dobson and Dean (1974) respectively.

The data were analysed according to the procedure and standards of Snedecor Cochran (1967). Differences in hormone concentrations among days postpartum were determined by ANOVA. Correlation was used to analyze the interrelationships among days postpartum and hormone concentrations.

#### **RESULTS AND DISCUSSION**

The changes in different levels of serum LH, FSH, estradiol-17ß and progesterone in postpartum dairy cows were depicated in Fig. The mean concentrations of LH and estradiol-178 in serum increased (P<0.01) gradually with days of postpartum in dairy cows. Serum LH and estradiol-178 increased from a mean of 0.55±0.01 ng/m and concentration 3.31±0.06 pg/ml at 1 day postpartum to 1.70±0.07 ng/ml and 5.70±0.03 pg/ml at 28 days postpartum. The rise in serum LH concentrations parallels the reported increase in bovine pituitary LH content during the first 30 days postpartum. However, the increase in pituitary LH content after parturition

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proportionally greater than the rise in plasma LH (Saiduddin *et al.* 1968).

LH (r=0.98; (P<0.01) Serum and estradiol-17β (r=0.87, (P<0.01) concentrations tended to increase with days postpartum. During late pregnancy, fetal cotyledons are rich source of estradiol-17ß (Gorski and Erb, 1959); however, within 24 hrs. following parturition, systematic estradiol-17ß concentrations decrease rapidly (Robertson, 1974). Follicular growth is detectable by 5 to 7 days postpartum (Callahan et al. 1971) which may explain the increasing estradiol-17ß concentrations with day postpartum. As estradiol-17ß concentrations increased, LH concentrations also tended to increase, It is likely that the increased serum LH levels observed during the first week postpartum in present study were atleast partially the result of positive feed back mechanism of estradiol on neuroendocrine centres which control LH synthesis and release. Similarly in heifers 12 hours after ovariectomy the LH secretion tended to increase following injection of estrogen (Hobson and Hansel, 1972).

FSH activity in serum was variable during the postpartum period. No difference was found among days for the maximum change in ron concentration. In dairy cattle plasma FSH concentrations have been reported to rise initially at postpartum, declining to the first oestrus (Dobson, 1978), or to exhibit no change (Schams *et al.* 1978).

Though concentrations of progesterone in serum showed a change with days postpartum, no concentration of progesterone in serum exceeded 0.5 ng/ml at any observation during the postpartum period, and therefore, differences are probably not important physiologically. These results are consistent with previously reported studies (Goodalo *et al.* 1978).

Neither differences among mean concentrations of the four hormone at 2 hourly collections on each day nor the day by time interaction were significant. This was expected since some cows exhibited increased concentrations of a hormone while other cows were decreasing at the same hour of sequential sampling

The present study suggests a significant positive correlation between LH and estradiol- $17\beta$  concentration from day 1 to day 28 of the postpartum.





113

## REFERENCES

Callahan C.J, Erb R.E, Surve A.H and Randel R.D. 1971. Variables influencing ovarian cycles in postpartum dairy cows. J. Anim. Sci. 33: 1053-1059.

Dobson H. 1978 Plasma gonadotrophins and estradiol during estrus in the cows. J. Reprod. Fert. 52:51-53.

- Dobson H and Dean P.D.G. 1974. Radioimmunoassay of estrone, estradiol-17 and 17β in bovine plasma during the estrous cycle and last stage of pregnancy. J. Endocr. 61:479-486.
- Goodalo W.S, Garverick H.A, Kesler D.J, Bierschwal C.J, Elmore R.G. and Youngquist R.S. 1978. Transitory changes of hormones in plasma of postpartum dairy cows. J. Dairy Sci: 61:740-746.

Gorski J. and Erb R.E. 1959. Characterization of estrogen in the bovine. Endocrinol. 64:707.

- Hobson W.C. and Hansel, W. 1972. Plasma LH levels after ovariectomy, corpus luteum removal and estradiol administration in cattle. Endocrinol. *91*: 185-190.
- Kanchev L.N, Dobson H, War W and Fitzpatrick, R.J. 1976. Concentrations of steroid in bovine peripheral plasma during the estrous cycle and the effect of betamethasone treatment. J. Reprod. Fert. 48:341-345.
- Robertson H.A. 1974. Changes in the concentrations of the unconjugated estrone, estradiol- 17β and estradiol- 17β in the maternal plasma of the pregnant cow in relation to the initiation of the parturition and lactation. J. Reprod. Fert. *36*:1-7.
- Saiduddin S.J, Riesen J.W, Tyler W.J. and Casida, L.E. 1968. Relationship of postpartum interval to pituitary gonadotrophins, ovarian follicular development and fertility of dairy cows. Wil. Res. Bull. 2:15-26.
- Schams D, Schallenberger E, Menzen Ch., Stangl K, Zottmier K, Hoffmann B. and Karg, H. 1978. Profiles of LH, FSH and progesterone in postpartum dairy cows and their relationship to the commencement of cyclic function. Theriogenology. 10(6):453-468.

Snedecor G.W. and Cochran W.G. 1967. Statistical Methods. 6th ed. The Iowa State Univ. Press. Ames.

- Stankov B.M., Uscheva A.A. and Kanchev L.N. 1986. The effect of the purity of the iodinated tracer on the specificity of a homologus assay of bovine FSH. Biochem. Internatis. 12:11-19.
- Stupnicki, R. and Madej, A. 1976. Radioimmunoassay of LH in blood plasma of farm animals. Endokrinologie Band 68. Heft 1, 6.13.

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# Chromosomal Chimerism and Gross Reproductive Anomalies In Freemartin Cattle

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## ABSTRACT

A total of 9 males or females from heterosexual twins were studied, out of which only two complete twin sets were available. A parallel relationship between the XX : XY cells in males and females of the twin sets was observed, which may be due to the predominance of one cell type in the twins with placental vascular anastomosis. Four adult females from this group were found to be sterile. The external and the per rectal examination revealed the abnormal development of the female genital tract and the cytogenetic screening revealed varying degree of XX ; XY chimerism. The females were confirmed as freemartins.

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The condition freemartinism in cattle was recognized many years ago as a type of infertility associated with anatomical defects of the female reproductive tract in heifers born co-twin to male (Marcum, 1974). Tendlor and Keller (1911) concluded that the choriovascular anastomosis of placentas was the primary cause of the condition. Ohno *et al.*, (1962) observed that the sex chromosome chimerism occurred both in freemartin cows and its male co-twin. A closs parallel relationship between the XX and XY sex chromosome in the male and its female members of the heterosexual twins was reported by Marcum *et al.*, (1972).

The present investigation was undertaken on 9 individual (Male and female) from heterosexual twins. The animals were examined for their chromosomal analysis, especially the percentage of XX and XY chromosome along with the gross reproductive disorder in cattle.

## MATERIALS AND METHODS

For heterosexual twin studies, a total of 9 individuals of different age groups were included

in the present study. Out of these, only two complete twin sets were available. Of the rest 5 animals, 4 females and 1 male partners of the twin sets were available, the other partners of the ses either died orwere disposed off, hence were not available for the study. The details regarding the animals as shown in Table 1. Out of the 6 chimeric females inlcluded in the prsent study, 4 were examined per rectally for the development of the reproductive organs. The blood samples from all the animals were cultured for obtaining the chromosomes, using simplified bovine lymphosyte culture method (Eldridge, 1982). The percentage of XX and XY cells were calculated for the individuals

## **RESULTS AND DISCUSSION**

The details regarding the animals studied, number of cells screened and per cent XX and XY cells in males and females are shows in Table 1.

## Examination of female reprodutive organs.

The per rectal examination of genital organs of female of twin set No. 1, which was about two years old revealed vagina, uterine body, uterine horns and small ovaries, however, their development was not proper as per her age. The heifer did not show any signs of estrus till the date of examination. The male partner of this set had satisfactory testicular development

The female No. 4 and 5 (Table 1), which belonged to the Holstein Friesian breed showed almost similar development of their reproductive organs. Externally clitoris was slightly enlarged. Per rectally they revealed small, shallow, vagine, cord like under developed uterine body followed by short uterine horns. The ovaries were not palpable. Both the heiters never showed any sign of estrus and were confirmed as freemartins.

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Ine remate No. 6 Which was about 4 years old non-descript heifer, was a typical freemartin. Externally she had enlarged phallus like clitoris, obstructing the passage of urine and the development of extra hairs around the vulva. Per rectal examination revealed short vagina, no palpable cervix, small cord like uterus without bifurcating horns and the absence of ovaries. It never showed any symptoms of estrus.

All the four chimeric females included in the present investigation were infertile, however, the actual mechanism that caused freemartinism remained elusive. Fechheimer *et al.*, (1963) proposed that the Y chromosome in the circulation of the female twin may be a factor in the etiology of the freemartin syndrome.

## Cytogenetic examination

The chromosomal studies of all the 9 animals (males and females) from heterosexual twin were carried out. The chromosomal chimerism (60, XX/XY) was observed in all the 9 animals. The details about the animals and the per cent chimerism are presented in Table 1, Fig. 1 indicates two adjacent chromosomal fields from male of the Jersey twin No. 1, showing both XX and XY chromosomes. The overall average percentage of XX cells observed was 42.08 per cent and that of XX cell was 57.9 per cent. The degree of chimerism which is expressed as the proportion of the XY cells, was higher in the twin female NO. 4 and 6 than in 3 and 5. The twin female No. 3 had the lowest XX cells which was 14 per cent.

EXTENSIVE STUDIES had been carried out on the proportion of the male (XY) and the female (XX) cells in both members of the twin pair (Kanagawa *et al.*, 1965; Herschler and Fechheimer, 1967; Yaday and Balakrishnan, 1985). In the present investigation 6 female members of the heterosexual twins revealed varying degree of chimerism from 37 to 86 per cent.

An interesting phenomenon observed was the occurrence of a parallel relationship between the ratio of XX : XY cells in the males and the females of the two twin sets included in the present study. It was significant to note in the two sets of the heterosexual males and females that the frequency of the XY cells in the male and the female of both the twin pairs was higher than the XX cells. In twinset No. 1, the frequency of the XY cells in the male and the female was 51.0 and 53.2 per cent and in the twin set No. 2, it was 62.0 and 59.0 per cent, respectively in male and the female. Similar parallel study of XX : XY cells could not be done in other sets, due to the non-availability of the twin partners.

Eldridge (1985) was of the opinion that there was some type of dominance of one cell type in twins with placental vascular anastomosis. The exact reason for this phenomenon has not been determined yet. The predominance of one cell type in the heterosexual twin sets has also been reported by Ohno *et al.*, (1962), Herschler and Fechheimer (1967), Moon ad Basrur (1967) and Yaday and Balakrishnan (1985).



Fig. 1: Chromosomal chimerism in a male partner of a heterosexual twin showing both XX and XY chromosome

## Table 1. Sex chromosome chimerism in heterosexual twins in cattle.

Twin	Breed	Age (Approx.)	Sex	No. of cells screened	Per cent XX	Per cent XY
1.	Jersey crossbred	2 years	Female Male	128 110	49 46.8	51 53.2
2.	Jersey crossbred	9 months	Female Male	107 122	41 38	59 62
3.	Jersey crossbred	1 year	Female Male	88 NA	14 0	86 0
4.	HF crossbred	6 years	Female Male	105 NA	63 0	37 0
5.	HF crossbred	3 years	Female Male	96 NA	30 0	70 0
6.	Non-descript	4 years	Female Male	115 NA	55 0	<b>45</b> 0
7.	Jersey crossbred	3 years	Female Male	NA 71	0 42	0 58
	Average				42.08	57.91

NA : Animals not available for the study

## REFERENCES

Eldridge, F.E. (1982) : Bovine lymphocyte methods. Mammalian chromosomes Newsletter, 23:(4176.

Eldridge, F.E. (1985) : Cytogenetics of livestock. AVI Publication Company Inc., Westport, Connecticut.

- Fechheimer, N.S.; Herschler, M.S. and Gilmore, L.O. (1963) : Sex chromosome mosaicism in unlike sexed cattle twins. Abst. in Genetics today. Proc. XI Int. Congr. Genetics, 1 : 265.
- Herschler, M.S. and Fechheimer, N.S. (1967) : Centric fusion of chromosomes in a set of bovine triplet. Cytogenet. Domest. Anim. 172.
- Kanagawa, H.; Kawata, K. and Ishikawa, T. (1965) : Chromosome studies on heterosexual twins in cattle.
  II. Significance of sexchromosome chimerizm chimerism (XX/XY) in early diagnosis of freemartin. Jpn. J. Vet. Res., 13 : 43.

Marcum, J.B. (1974) : The freemartin syndrom. Anim. Breed. Abstr., 42 : 227.

Marcum, J.B. (1974) : The freemartin syndrom. Anim. Breed. Abstr., 42 : 227.

Marcum, J.B.; Lasley, J.F. and Day, B.N. (1972) : Variability of sex chromosome chimerism in cattle from heterosexual multiple births. Cytogenetics, 11: 388.

Moon, Y.S. and Basrur, P.K. (1967) : Proc. 10th Canad. Fed. Biol. Soc. 43.

Ohno, S.; Trujillo, J.K.; Stenius, C.; Christian, L.C. and Teplitz, R.L. (1962). Possible germ cell chimer as among new born dizygotic twin calves (Bos taurus). Cytogenetics '1 : 258.

Tandlor, J. and Keller, K. (1911): The chorion in differently sexed twin pregnancy of the cow and the morphology of the female animals which result. *Dtsch. Tieraerzt. Wochenschr., 19: 148.* 

Yadav, B.R. and Balakrishnan, C.R. (1985) : Parallelism of XX/XY chimerism in cattle and buffalo multiple birth. Indian J. Dairy Sci., 38 : 62.

117

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# Studies on Uterine Bacterial Microflora in Buffalo with Dystocia

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## ABSTRACT

Uterine fluid from 15 buffaloes suffering from dystocia were collected after removal of the dead foetuses and subjected to bacterial culture. Bacterial growth was achieved from 14 samples (95 %).The microflora belonged to 5 different genera. Among the principal isolates, E.coli (5), S. aureus (4), Pseudomonas aeruginosa (3), Streptococcus SPD. (2)and Corvnebacterium progenes (1) were predominant. In vitro drug sensitivity results revealed that gentamicin was the most effective drug (80 %) against the bacterial isolates while erythromycin, penicillin and ampicillin were totally ineffective.



Post-partum bacterial endometritis is a common cause of infertility and economic losses as it delays the onset of post-partum ovarian activity and prolongs inter-calving intervals (Erb et al., 1958). Buffaloes with dystocia often end up with metritis or pyometra. Bacterial toxins of uterine origin could be involved with the high death rate (upto 55 %) reported in buffaloes suffering from dystocia (Nanda et al., 1991). Hussain et al. (1990) has reported a number of bacterial isolates from uteri of operated cows and were associated with delayed uterine involution. The present study was undertaken to know the type of uterine bacterial microflora and the most effective antibiotic in buffaloes with dystocia.

### MATERIALS AND METHODS

### **Collection of specimens**

Fifteen buffaloes suffering from dystocia, either due to foetal malpresentation or uterine torsion were presented for the treatment. These cases were of 12 to 72 h duration at the time of presentation for treatment. Dystocia was relieved either by rolling the dam for uterine detorsion or by caesarean section. The foetus was dead in each case at the time of treatment.

Immediately after the removal of dead foetus, the uterine fluid samples were collected either through vagina or from the caesarean site in a total aseptic manner in sterilized glass vials.

## Isolation and identification of the organisms

Immediately after the fluid collection, it was streaked onto 10% sterilized sheep blood agar plates. These plates were then incubated at 37 c for 24-48 h. Colonies appearing on the plates were studied for their morphology and a representative colony from each plate was stained by Gram's method. Further, identification and characterization of the organisms was made on the basis of their morphological, cultural and biochemical tests (Carter, 1984).

#### In vitro drug sensitivity test

In vitro drug sensitivity test of all the bacterial isolates was carried out against nine antibiotics (Table 1) by employing standard disc diffusion technique (Bauer *et al.*, 1966) on Mueller-Hinton agar plates and the zone of inhibition was measured in millimeters.

## **RESULTS AND DISCUSSION**

From a total of 15 specimens subjected to bacteriological studies, 14 yielded the growth of bacteria while one specimen was found to be sterile. Pure growth of bacteria was seen in 13 specimens of uterine fluid while one yielded a mixed growth of *E Coli* and *S. aureus*. The details of isolation are presented in Table 1.

Pathogenic organisms cause uterine inflammation, denudation of its mucosa and increase its secretions, thus altering uterine environment leading to repeat breeding. *Escherichia coli, Pseudomonas aeruginosa* and streptococci have been isolated from the uterine swabs of repeat breeding buffaloes (Singh, 1979). In uteri of cows with retained placenta, *Proteus* spp. and *Clostridium* spp. were also present in addition to the organisms isolated in the present study (Hussain *et al.* 1990).

In vitro drug sensitivity test against 9 different drugs revealed that gentamicin was the most effective drug against all the isolates followed by chloramphenicol, co-trimoxazole and furazolidone (Table 1). All the bacterial isolates were completely resistant to penicillin, ampicillin and erythromycin. *Pseudomonas aeruginosa*  was found to be the most resistant organisam which was totally (100%) resistant to 6 of the nine antibiotics tested. There seems to be no information available for comparison of the microbial picture of the uterine fluid and their sensitivity to antibiotics after relieving dystocia in buffaloes. Since a vast variety of organisms were isolated from the uterine fluid and that no single antibiotic was found consistently effective, it is suggested to treat puerperal uterine infections after properly ascertaining the drug of choice.

Table 1. Isolation and *in vitro* drug sensitivity of the organisms isolated from uterine fluid of buffaloes with dystocia

S.No.	Name of the bacteria	Total No.	-	4	lo. of	of isolates found sensitive to effective antibiotics					
			G	С	Co	FR	Т	E	A	Р	S
1.	Escherichia coli	5	5	4	2	1	2	-	-	-	1
2.	Staphylococcus aureus	4	4	З	1	2	1	-			-
3.	Pseudomonas aeruginosa	3	2	1	1		-		-	-	-
4.	B-haemolytic streptococci	2	2	2	2	2	-	-	-	-	
5.	Corynebacterium pyogenes	1	1		1	1	1		-	-	1
	Total	15	14	10	7	6	4	0	0	0	2

G = Gentamicin (10 mcg) C = Chloramphenicol (30 mcg) Co = Co-trimoxazole (25 mcg) FR = Furazolidone (100 mcg) S = Streptomycin (10 mcg)

- T = Tetracycline (30 mcg)
- E = Erythromycin (15 mcg)
- A = Ampicillin (10 mg)
- P = Penicillin (10 units)

## REFERENCE

- Bauer, A.W., Kirby, W.M.M., Sherris, J.C. and Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disc method. American J. Clin. Path. 46: 496.
- Carter, G.R. 1984. Diagnostic Procedures in Veterinary Bacteriology and Mycology. Fourth edition. Charles C. Thomas Publisher, Springfield, Illinois.
- Erb, R.E., Hinze, P.M. Gildow, E.M. and Morrison, R.A. (1958). Retained fetal membranes. The effect on prolificacy of dairy cattle. J. Am. Vet. Med. Assoc. 133 : 489-496.
- Hussain, A.M., Daniel, R.C.W. and Boyle, D. (1990). Postpartum uterine flora following normal and abnormal puerperium in cows. Theriogenology 34 : 291-302.
- Nanda, A.S., Sharma, R.D. and Nowshahari, M.A. (1991). The clinical outcome of different regimes of treatment of uterine torsion in buffaloes. Indian J. Anim. Reprod. 12: 197-200.
- Singh, R.B. 1979. Studies on infectious causes of repeat breeding and their response to treatment in bovines. Ph.D. Thesis, submitted to Punjab Agric. Univ., Ludhiana.

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# Effect of Intrauterine Therapy on Postpartum Endometritis in Dairy Animals

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## ABSTRACT .

Intrauterine therapy constituting different drugs was tried in postpartum endometritis in Karan Swiss (123).Karan Friesian (91).Sahiwal cows (7) and Murrah buffaloes (23). The drugs tried were Metrijet (n=17), Terramycin liquid (n=53), Betadine (n=22), Vetran-LA (n=81) and Furea(n=7) and took an average of 16, 18, 20, 18 and 19 days for declaring the animals clinically cured. The study suggested that the therapeutic effect of the drugs amongst different groups was not significantly different in cases of postpartum endometritis in dairy animals including crossbreds, zebu and buffaloes.

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Various therapeutic agents have been advocated and tried for the treatment of endometritis in dairy animals in general and buffalo in particular following parturition. It is still not advisable to prescribe with confirmity that a particular line of treatment using a specific preparation is superior to other line of treatment for curing various degrees of endometritis. However, the objective of all the treatments used is to stimulate the uterine muscles through higher vascularity either by increasing the blood supply or to create a healthy and sterile environment in the uterus by checking and controlling the uterine infection. In the present study both types of treatments were tried in case of endometritis devoloped during normal course or followed by retained foetal membrane (RFM) accompaning premature births, still born calves and dystokia. The use of Betadine increases the flow of blood circulation to the uterine endometrium and creates antiseptic environment, whereas, terramycin liquid, nitrofurazon, vetran-LA and metrijet check the infections in the uterus.

Uterine infrctions terminating in endometritis and various forms of metritis are likely to follow abnormal parturitions such as abortion, dystokia and lacerations (Roberts, 1976). These pathological conditions are generally associated with delayed involution of uterus and postpartum ovarian activity leading to prolonged postpartum anestrum. The present trial envisages the use of various therapeutic agents in the cases of endometritis and metritis followed by RFM in cows and buffaloes after parturition and to check these genital infections in order to have successful breeding results with shortest possible calving interval.

### MATERIALS AND METHODS

The animals belonged to the National Dairy Research Institute herd (NDRI), Karnal, Harvana and were crosses of Brown Swiss and Zebu (KS), Holstein Friesian and Zebu (KF) Sahiwal cows and Murrah buffaloes. These animals were closely monitored for various reproductive disorders such as abortion, still birth, retained foetal membranes and dystokia occured during puerperal and postpartum periods followed by endometritis and metritis till they are clinically cured after giving intra- uterine administration of some therapeutic preparations such as Metrijet (Intervet-Holland, n=17), Terramycin liquid, Pfizer, n=53) Betadine solution (Wockhardt, n=22) Vetran-LA (Vets-pharma, n=81 and Fures bolus (SK&F, n=71).

The animals retained foetal membranes following 12 hours of calving were subjected to detailed gynaecological examination. Uterine infection with a watery to cloudy or purulent vaginal discharge alongwith incomplete uterine involution was considered as the sign of endometritis/metritis.

All the drugs were prepared freshly in sterile glass distilled water and were introduced in the

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uterus with the help of a sterilized uterine catheter. The medicines were repeated after three days till the animals were declared clinically cured with not more than 5 applications. Dose schedule : The drugs were administered as 10 ml in primiparous and 30 ml in pluriparous animals each time except metrijet given in readymade full syringe. Vetran-LA and Furea both were administered with 2 and 4 tablets by dissolving in 10 ml and 30 ml water, respectively.

The uterine discharge of these animals was routinely checked. The animals were declared clinically cured only when the uterine discharge was found clear after treatment.

## RESULTS AND DISCUSSION

No significant effect difference among different treatment groups was observed and required on an average of 16-20 days for a clinical cure of all animals including that of crossbred cows. However, Metrijet treatment took less time with an average of 16 days in comparison to other treatment drugs. A maximum period of 20 days (range of 8-50 days) were taken in animals treated with Betadine solution.

Since iron and copper being the major circulatory elements and were at a lower profile on the day of calving, perhaps, led to the occurance of abortion, still birth and RFM leading to endometritis (Jain 1993). The use of lugol's iodine solution (Agarwal and Pandit 1991; Gupta et al. 1989; Nikhara et al. 1977). Broad stectrum antibiotics and Fures bolus (Dutta and Dugwekar 1981 and Sinha and Khan 1989) in cases of endometritis followed by RFM, did not reveal significant difference of period for anv establishing a clinical cure after intrauterine administration. A similar finding was also observed in the present study. Results further revealed that there was no special advantage in using medicines which are costly and also not easily available, had difficulty in administration and which might become less sensitive after repeated use in cases of endometritis as well as metritis.

#### REFERENCES

- Agarwal, R.G. and Pandit, R.K. (1991). Practical approach to therapeutic management of some commonly occurring reproductive disorders leading to infertility in bovines. Vet. 3: 219-225.
- Dutta, J.C. and Dugwekar, Y.G. (1981). Therapeutic value of broad spectrum antibacterial drugs in the treatment of placental retention in cows. I.J.A.R. 1: 116.
- Gupta, R.C.; Krishnaswamy, A. and Sinha, A.K. (1989). Effect of lugol's solution on the bovine endometrium. I.J.A.R. 10: 147-148.
- Jain, G.C. (1993). Circulatory levels of minerals during puerperal and postpartum periods in crossbred cows. Int. J. Anim. Sci. 8: 167-169.
- Nikahara, T.; Domaki, I. and Yamauchi, M. (1977). Local effect of intrauterine in fections of iodine solution on the life span of the corpus luteum of the cow. J. Reprod. Fert. 36: 425-435.
- Roberts, S.J. (1976). Veterinary obstetrics and genital diseases. Edward Bros, Inc. Ann. Arobos, Michingan USA.
- Sinha, N.K. and Khan, B.U. (1989). Effect of therapeutic preparations on postpartum conception rate in ewes, I.J.A.R. 2: 188-189.

# Studies on serum cholesterol levels of anoestrus cows treated with GnRH-analogue (Receptal)

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## ABSTRACT

A highly significant difference in the levels of serum cholesterol was observed in treatment group-I and II 256.98  $\pm$  19.61 Vs 236.44  $\pm$  29.04, 252.51  $\pm$  22.04 Vs 236.44  $\pm$  29.04 mg. per cent (before treatment) as compared to control group animals, while it was significant on 10th and 17th day in group-I and on 17th day in group-II (P < 0.01). No significant difference in the levels of serum cholesterol was observed in control and treatment group-I on 1st day and in group-II on 1st and 10th day. Oestrus induction was 87.5, 50.0, 0.0 percent in Group-I, II and control group respectively.



Anoestrus is the condition where there is absence of reproductive cycles. Such animals have smooth inactive gonads without presence of any palpable folliculor or luteal activity (Pargaonkar, 1978). Cows though apperantly normal, appear weak, get some feed and fodder but fail to manifest oestrus cycle at regular intervals. Steroid hormone levels can be judged by knowing serum cholesterol level because cholesterol acts as a precuser for the synthesis of steroidal hormones. Therefore, the aim of present investigation was to study serum cholesterol levels in anoestrus cows treated with GnRH-analogue (Receptal-Hoechst India Ltd).

## MATERIALS AND METHODS

In all 24 healthy cows with anoestrus condition were selected and divided into three groups (two treatment and one control group). Each group comprised of 8 cows. Cows from group-I were treated with Injection Receptal 5 ml. (2.210 mg. GnRH-analogue) intramuscularly in the neck region as single dose treatment. Cows from group-II were treated with Injection Receptal 2.5 ml. (0.105 mg. GnRH-analogue) injected in the vulval submucosa region as single dose treatment. Cows from group-III were kept as untreated control. Blood from every cow was collected from jugular vein before treatment and following treatment (1st, 10th and 17th day). Serum was separated and stored in glass vial after adding one drop of Toulene per vial. Serum vials were stored at -20°C in deep freeze. The serum cholesterol was estimated by one step method (by Kit method) of Wybenga and Pileggi (1970). The compiled data was analysed by using paired 'T' test as per Panse and Sukhatme (1986).

## **RESULTS AND DISCUSSION**

Details of estimated mean values of serum cholesterol are given in Table-1. Oestrus induction was 87.5, 50.0, 0.0 percent in Group-I, Il and control group respectively.

**Group-I:** Serum cholesterol levels in the treated animals were observed as  $256.98 \pm 19.61$  Vs  $236.44 \pm 29.04$  (before treatment),  $159.82 \pm$ 10.78 Vs  $156.06 \pm 6.88$ ,  $120.16 \pm 8.43$  Vs  $136.63 \pm 3.68$  and  $106.31 \pm 6.81$  Vs 163.82 $\pm 15.61$  mg. per cent on 1st, 10th and 17th day following treatment as compared to control group animals. Mean serum cholesterol levels in experimental animals were ranging between  $106.31 \pm 6.81$  to  $256.98 \pm 19.61$  Vs 136.63 $\pm 3.68$  to  $236.44 \pm 29.04$  mg. per cent (Av.  $160.88 \pm 11.40$  Vs  $174.23 \pm 13.80$  mg. percent) as compared to control group, which was found to be within the normal range.

Highly significant difference in the level of serum cholesterol was observed before treatment in treatment group-I and control group (P < 0.01), while it was significant on 10th and 17th day (P < 0.01). However, non-significant difference in the level of serum cholesterol was observed in control and treatment group-I on 1st day.

Present findings are lower than those of earlier reports of Prasad et al. (1984), Verma
et al. (1984), Pareek and Deen (1985) and Dhaliwal and Sharma (1990), as 224.44, 201.6  $\pm$  11.89, 181.61 and 256.24  $\pm$  15.17 mg. per cent respectively. Present findings are higher than those of earlier reports of Murtza et al. (1978), 129.41  $\pm$  6.96 mg. per cent in empty dry cows, Mane (1991), 121.58 mg. per cent in Red Kandhari post-partum anoestrus cows after treatment.

**Group-II:** Serum cholesterol levels in treated animals were observed as  $252.51 \pm 22.04$  Vs  $236.44 \pm 29.04$  (before treatment),  $157.89 \pm$ 14.64 Vs  $156.06 \pm 6.88$ ,  $141.63 \pm 10.20$ Vs  $136.63 \pm 3.68$  and  $138.77 \pm 10.75$  Vs  $163.82 \pm 15.61$  mg. per cent on 1st, 10th and 17th day following treatment as compared to control group animals.

Mean serum cholesterol levels in experimental animals were ranging between 138.77  $\pm$  10.76 to 252.51  $\pm$  22.04 Vs 136.63  $\pm$  3.68 to 236.44  $\pm$  29.04 mg. per cent (Av. 172.7  $\pm$  14.41 Vs 174.23  $\pm$  13.80 mg. per cent), which was found to be within the normal range.

Highly significant difference in the levels of serum cholesterol was observed before treatment in treatment group-II and control group, while it was significantly on 17th day (P < 0.01). However, non-significant difference in the level of serum cholestrol was observed in control and treatment group-II on 1st and 10th day.

Present findings are in agreement with those of Bagal and Kadu (1988),  $173.20 \pm 9.88$ mg. per cent in crossbred cows. Higher percentages of cholestrol levels are reported by Prasad *et al.* (1984), Verma *et al.* (1984), Pareek and Deen (1985) and Dhaliwal and Sharma (1990), 224.44, 202.6  $\pm$  11.89, 181.61 and 256.24  $\pm$  15.17 mg. per cent

It was observed that serum cholesterol level dropped down continuously after treatment in both the treatment group cows. which menifested heats, where as it dropped down upto 10th and thereafter it increased in the control group cows, which remained anoestrus through-out the experimental period. This suggested the effect of GnRH-analogue on the serum cholesterol levels of cyclical and non-cyclical cows. Further detailed observations with more number of animals are necessary to confirm the findings.

Sr.	Group of animals	1.1.1	Serum cholesterol Following						
		Before							
		-	1st day	10th day	17th day				
1	Treatment	256.88**	159.82	120.16	106.31				
	group-l	±19.61	±10.76	±8.43	±6.81				
2	Treatment	252.51**	157.89	141.63	138.77				
	group-II	±22.04	±14.64	±10.20	±10.76				
3	Control	236.44**	156.06	136.63	163.82 <sup>1</sup>				
	group	±29.04	±6.88	±3.68	±15.61				

Table 1: Showing the details of Mean Serum cholesterol (mg. per cent) before and following treatment (Group-I, and II) with Receptal (GnRH-analogue) compared with controls

Highly significant at (P<0.01).

#### REFERENCES

- Bagal, S.B. and Kadu, M.S. (1988) Effect of GnRH-analogue (Buserelin) on some blood constituents in post-partum crossbred cows. Indian J.Anim. Reprod. 2(2): 85-87.
- Dhaliwal, G.S. and Sharma, R.D. (1990) Cholesterol levels following induction of oestrus with PGF<sub>2</sub> alpha in buffaloes using two routes of administration. Indian Vet. J. 67(8) 719-723.
- Mane, K.S. (1991) Studies on efficacy of PGF<sub>2</sub> alpha (analogue) to induce cestrus in post-partum ancestrus cows. M.V.Sc. Thesis, submitted to MAU, Parbhani.
- Murtza, K; Pandey, M.D. and Rawat, J.S. (1978) Serum enzymes and serum cholesterol in Hariyana cattle under different physiological status. Indian Vet. J. 55(12): 958-962.
- Panse, V.G. and Sukhatme, P.V. (1986) Statistical methods for Agricultural Workers. Published by: I.C.A.R., New Delhi.
- Pareek, P.R. and Deen Anim. (1985) Changes in blood profile from ante-partum, parturition to post-partum period in anoestrus and normal reproduction Rathi cows. Indian J.Anim.Reprod. 6(2): 33-36.
- Pargaonkar, D.R. (1978) Studies on bovine gynaecology, fertility problems of reproduction in native and crossbred. Ph.D. Thesis, submitted to PKV, Akola.

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- Prasad, R.S; Kharche, K.G. and Shrivastava, O.P. (1984) Studies on blood glucose, cholesterol and total leucocyte count in anoestrus crossbred cows. Indian J. Anim. Reprod. 4(2): 10-14.
- Verma, R.P; Bhaji, H.K; Gary, R.C. and Mishra, R.R. (1984) Biochemical studies on reproductive status of Hariyana cows. Livestock Advisor. /X(VII) : 29-34.
- Wybenga and Pilaggi (1970) Estimation of serum cholesterol (by kit method) Span Diagnostic Pvt. Ltd., Udhana (Surat).

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# Treatment of Ovarian cysts in Cows

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## ABSTRACT

Twenty one cross bred cows diagnosed as having ovarian follicular cysts from rectal palpation of the ovaries were selected for this study. Animals were treated at random with either intramuscular injection of 200 mcg GnRH, or intramuscular injection of 3000 i.u. HCG. Differences in clinical responses and subsequent fertility between two treatment groups were not significant. Fifty percent cows given HCG and 62.5 percent cows given GnRH resumed overian activity within a period of 15 to 31 days with subsequent conception.

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Cystic ovarian disease is one of the important causes of infertility and affects a high proportion of dairy cows, extending the intercalving periods and leads to economic losses to the animal owners (Bartiett et al., 1986). In most of the cows where cysts developed 20-30 days post-partum, cysts regress spontaneously within 10 days or so without affecting fertility (Morrow et al., 1966). If the cysts persist for longer duration, an efficient method of treatment is required. Various methods of treatment includes manual rupture (Dawson, 1956); administration of LH/HCG (Nakao et al., 1978; Bugalia and Kohli, 1981; GnRH (Elmore et al., 1975; Kesler et al., 1979) with varied results. In the present study attempts were made to treat the cystic ovarian disease in cows with human chorionic gonadotropin or GnRH.

#### MATERIALS AND METHODS

Twenty one cross bred parous cows diagnosed as cases of cystic ovarian disease, at the Veterinary Clinics, College of Veterinary, Sciences, Pantnagar, were included in this study. All the animals were examined per-rectally twice a week continuously for a period of one month to observe any structural changes on ovaries.

On the basis of history of irregular estrus for the last 2-7 months and soft enlarged cysts on the ovaries as reveald on rectal palpation, the cases were diagnosed as follicular cysts. Findings were recorded at the time of diagnosis as to the site, size, number and characteristics of the cyst(s) for comparison during subsequent examinations and for evaluation of treatment. Ten animals were administered intramuscularly with 3000 i.u. HCG (Chorulon - Intervet International) and 8 animals were injected intramuscularly with 200 mcg GnRH (Receptal - Hoechst India Ltd). Three animals served as control and were administered saline only. Following treatment all animals were observed clinically and examined per-rectally daily starting from days 3 of treatment and rsponse to therapy was evaluated. Final response to treatment was recorded as positive if a normal estrous cycle followed or the animal conceived. A negative response was recorded if no change in the cyst structure could be observed. Subsequent estrus behaviour and fertility were also recorded for cows responding. positively.

## RESULTS AND DISCUSSION

Differences in clinical response and subsequent fertility between two treatment groups were not significant. After the administration of HCG or GnRH the ovarian cysts regressed within 8 days in most of the animals. Five out of the 10 animals treated with HCG, responded with manifestation of normal estrus sign within a period of 17 to 31 days. They were artificially inseminated and all of them conceived. In 3 cows no effect was observed and in 2 animals cysts reappeared and estrous cycle remained irregular. Of the 8 cows treated with GnRH, 5 cows responded satisfactorily leading to appearance of estrus within 15 to 30 days and subsequent conception. One cow not responded with GnRH treatment and in two cows cysts reappeared following regression. No change was observed in the control group.

Following 3000 i.u. of HCG administration the recovery rate was 50 percent and subsequent conception within 17 to 31 days. Several other workers (Laing, 1979; Tanabe and Brofee, 1982; Koppinen *et al.*, 1984) have also used LH/HCG and achieved 30-58% conception rates in the first observed estrus following treatment. A conception rate of 62.5 percent was achieved following 200 mcg GnRH administration. This conforms well with the report of Elmore *et al.*, 1975; Garverick., *et al.*, 1976, Kesler *et al.*, 1979 who reported that GnRH caused recovery in 60-100 percent cases within 19-35 days with 49-65 percent conception rate to first estrus.

Cyst regressed in only one cow and in other no effect was observed of repeated administration of HCG in the cows which did not respond or in which cysts reappeared after the first HCG injection. However, a positive response was achieved by second injection of GnRH in cows which had not responded or in which cyst reappeared with first GnRH injection. This is understable because of small molecular size, GnRH is not likely to stimulate an immune response as is occasionally produced by exogenous HCG (Greenwald, 1970). Repeated GnRH therapy should, therefore, not result in refractoriness nor should it likely result in anaphylactic reactions.

This study demonstrated that there were no significant differences in clinical response when 200 mcg GnRH or 3000 i.u. HCG were used to treated the cows with ovarian cysts.

## REFERENCES

- Bartlett, P.C.; Ngategize, P.K.; Kaneene, J.B.; Kirk, J.H.; Anderson, S.M.; Mather, E.C. (1986). Cystic follicular disease in Michigan Holstein Friesian cattle: incidence, descriptive epidemiology and economic impact. Priventive Veterinary Medicine 4:15-33.
- Bugalia, N.S. and Kohli, I.S. (1981). Comparative efficacy of proluton depot, gonadotrophin LH and potassium iodide in nymphomaniac Rathi cows. Haryana Agricultural Univ. J. Res. 11:575-577.

Dawson, F.L.M. (1957). Bovine cystic ovarian disease - a review of recent progress. Br. Vet. J. 113:112-133.

- Elmore, R.C.; Bierschwal, C.J.; Youngquist, R.S.; Cantley, T.C.; Kesler, D.J. and Carverick, H.A. (1975). Clinical responses of dairy cows with ovarian cysts following treatment with 10,000 i.u. HCG or 100 mcg GnRH Vet Med. Sm. Anim. Clin. 70:1346-1349.
- Garverick, H.A.; Kesler, D.J.; Cantley, T.C.; Elmore, R.G.; Youngquist, R.S. and Bierschwal, C.J.; (1976). Hormone response of dairy cows with ovarian cysts after treatment with HCG or GnRH. Theriogenology. 6:413-425.
- Greenwald, G.S. (1970). Development of ovulatory refractoriness in the rabbit to cyclic injections of Human chorionic Gonadotropin, Fertil and Steril 21:163-168.
- Kesler, D.; Garverick, H.A.; Elmore, R.G.; Youngquist, R.S. and Bierschwal, C.J. (1979). Reproductive hormones associated with the ovarian cyst response to GnRH. Theriogenology 12:109-114.
- Koppinen, J; Vesanen, M. and Alanko, M. (1984). Ovarian cysts in dairy cattle some aspects of diagnosis, treatment with GnRH and HCG and subsequent milk progesterone values. Nordisk Veterinaemedicin 36:26-31.
- Laing, J.A. (1979). Fertility and infertility in domestic animals. 3rd edn. Bailliere Tindal, London.
- Morrow, D.A.; Roberts, S.J.; McEntree, K. and Gary, H.G. (1966). Post-partum ovarian activity and uterine involution in dairycattle. J. Amer. Vet. Med. Assoc. 149:203.
- Nakao, T.; NUmato, Y.; KUbo, M. and Yamauchi, S. (1978). Treatment of cystic ovarian disease in dairy cattle. Cornell Vet. 68:161-178.
- Tanabe, T.Y. and Brofee, R.D. (1982). Treatment of cystic ovarian follicles indairy cows. With chorionic gonadotropin. Theriogenology 18:497-512.

IJAR. 15(2), 1994; 127-130

# Efficacy of certain Non-hormonal and Hormonal drugs on estrus induction in Post-partum anestrous buffaloes.

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## ABSTRACT

A field clinical trial was undertaken to induce estrous in rural Post-partum. anestrous buffaloes by the administration of certain non-hormonal drugs like clomiphene citrate ('Fertivet'), 'Prajana', Lugol's iodine solution paint to os cervix, 'Cvclomin-7'bolus and hormonal preparations like progesterone and Gonadotrophic releasing hormones (Gn-RH 'Receptal') injections.

Among the non-hormonal drugs tried clomiphene citrate ('Fertivet') induced estrus in 80.00 per cent of the treated animals with a mean interval of 21 days and subsequently a conception rate of 87.50 per cent was obtained by first insemination itself. The response with other non-hormonal drugs on estrus induction was in a descending order.

Among the hormonal preparations, progesterone treatment could produce highly encouraging estrous induction rate of 80 per cent with a mean period of 7.43 days and also a good conception rate of 75 per cen.

## \_\_\_\_X\_\_\_\_X\_\_\_\_

Post-partum anestrous condition is one of the most common reproductive disorders encountered in rural buffaloes leading to prolonged inter-calving periods, reduced milk production and thus affecting greatly the economy of our farming community. This reproductive disorder was reported to be more acute in buffaloes than in cows.

The incidence of anestrous condition in buffaloes was reported to range between 13.33 and 45.57 per cent by several investigators in this field (Luktuke *et al.*, 1973, Nagaraju, *et al.*, 1991, Umesh, 1992).

Several factors like insufficient release or production of gonadotrophic hormones and supression of release of gonadotrophic hormones due to under feeding, low hormonal profile etc., in rural cows and buffaloes were found to be the cause of anestrous condition in these animals 1969). Several investigators (Moustgaurd, succeded inducing heat by usina in non-hormonal and hormonal drugs singly or in combination. (Porwal et al., 1976, Rao and Rao, 1984 and Satish Kumar, 1990).

A field clinical trial was undertaken to study the efficacy of certain non-hormonal drugs like clomiphen citrate ('Fertivet') Prajana Capsules (Herbal preparation), Lugol's lodine paint, 'Cyolomin-7' Bolus and hormonal preparatins like progesterone ('Duraprogen') and Gonodotrophic hormone Releasing Hormone (Gnrh, 'Receptal') in inducing estrous in rural post-partum anestrous buffaloes with an aim to reduce the calving interval by way of initiation of early post-partum estrous.

## MATERIALS AND METHODS

A total of 140 rural non-descript post-partum anestrous baffalo cows with apparently in good condition brought to the college campus veterinary hospital and Ambulatory veterinary clinic, Mylardevpally with a history of Post-partum anestrum of more than 180 days, were included in this study. These experimental animals were subjected to the rectal examination on three occasions at weekly intervals for the conformation of true anestrous condition. The buffaloes were randomly kept under 7 groups of twenty animals each comprising of 6 groups of treatment and one control group.

Group-1 anestrous buffaloes were orally administered with one tablet of 'Fertivet' (Arex

<sup>\*</sup> Part of M.V. sc., Thesis (Animal Reproduction) submitted by the Senior Author to A.P. Agricultural University, Hyderabad 500 030.

laboratory, Bombay) dissolved in 500 ml of distilled water daily for five consecutive days with a prior drenching of 125ml of one percent copper sulphate solution.

Group-II animals were treated with 'prajana' capsules (Indian Herbs) at the rate of 3 capsules orally in the form of an electuary for three consequtive days.

Group-III animals were administered with two micromineral buluses 'Cyclomin-7' (Alved) orally along with the feed once in every three days for a period of two weeks.

Group IV Buffaloes were treated with a paint consisting of one percent lugol's iodine solution to the os cerwix for three times at an interval of three days.

Group V buffaloes were treated with progesterone ('Duraprogen', Unichem Laboratory) at a daily dose rate of 50mg intramuscularly to each animal for a period of five consecutive days.

Group VI animals received intramuscular injection of 5 ml synthetic gonadotrophic releasing hormone (Receptal, Hoechst).

Group VII animals received no treatment as they served as controls.

Detection of estrous in all the groups of animals was carried out on the basis of the exhibition of behavioural symptoms of heat coupled with the confirmation of the same by the rectal palpation. The animals failed to exhibit estrous signs within a stipulated period of 45 days post-treatment were considered to be non-responsive to the drug.

## RESULTS AND DISCUSSION

From the results observed in this study, administration of clomiphene citrate ('Fertivet') induced estrous in 16 of the 20 treated buffaloes (80 percent) with a mean interval of 21 days from the last day of treatment. Werheas, in the control group of animals, estrous, induction was observed ony in 30 per cent animals with a mean interval of 30.16 days (Table). Similar results were reported by several workers in this field (Deshpande *et al.*, 1976; Hukeri *et al.*, 1979, Dugwekar *et al.*, 1980 and Reddy *et al.*, 1990). The induced ostrous response of 'Fertivet' was reported to be due to its stimulatory action on the hypothalamus to release the Gonadotrophic releasing hormone (Gn-RH) and

its action on the pituitary gland to secrete and release the Gonadotrophic hormones particularly the Leuteinising hormone (Deshpande et al., 1976 and Aminudeen and Tanwar 1988). The longer interval of induced estrus effect of 'fertivet' administration (21 days) as observed in this study in comparison to the results obtained by other workers might be due to the delayed response of the drug in rural buffaloes fed on low energy diet and its subsequent delay in the secretion of gonadotrophic hormones by the pituitary gland (Moustguard, 1969). The mean conception rate of 87.50 percent recorded in this study with 'Fertivet' induced estrous animals is in close agreement with the reports of Varma and Khache (1983); Reddy et al., (1990) and kadu and Chede (1992).

'Prajana' Capsule (Indian Herbs), a herbal estrous Inducer, Induced estrus in 15 of the 20 animals (75 per cent) treated in this study with a mean interval of 16.11 days and 13 of the 15 induced estrous buffaloes settled to first insemination itself (Table). The results observed in this study are in agreement with the observations made by Naresh Chand (1974) who has illustrated the action of 'prajana' similar to that of synthetic estrogen in the induction of estrous. The estrous induction and conception rates noticed in this investigation are in line with the reports of Porwal *et al.*, (1976) and Patil *et al.*, (1983)

Feeding of anestrous buffaloes with 'cyclomin-7' boluses (Alved), а mineral preparation, evinced estrous in 55 per cent of the treated animals with a mean interval of 23.63 days and a conception rate of 81.81 per cent was recorded in this study (Table). This encouraging results of 'cyclomin-7' is an indication of the stimulatory effect of the trace elements contained in it in improving the reproductive efficiency in anestrous animals. The results obtained in this study are in line with " the observations of several researchers in this field (Porwal et al., 1976; Singh et al., 1978; Satish Kumar, 1986 and Birade et al., 1992).

Application of paint consisting of one per cent lugol's iodine solution to the os cervix initiated estrous behaviour in 10 out of 20 buffaloes treated in this study with a range of 5 to 28 days post-treatment. Among the induced estrous animals 70 per cent conceived to the first insemination itself (Table). The observations noticed in this study are in confirmation with the findings of Porwal *et al.*, (1976); Patil *et al.*, (1983) and Satish Kumar (1990). The action of Lugol's iodine in the induction of estrus was thought to be due to either stimulatory effect on the Hypothalamus (Patil *et al.*, 1983) or by the release of uterine leuteolytic factor acting via the utero-ovarian and uteropituitory-ovarian pathway (Varma and Kharche, 1983). The variation in the reports of the efficacy of Lugol's iodine in inducing estrous might be due to the proportion of iodine concentration, duration of application etc. to the os cervix.

Estrous could be induced with a mean period of  $7.43\pm0.32$  days in 80 per cent of the buffaloes treated in this study consequent to Instramuscular administration of 50 mg progesterone ('Duraprogen', Unichem) daily for five consecutive days (Table). The results observed in this study are in confirmity with the findings of Singh and Singh (1986). Administration of progesterone for a period of 5 days and its subsequent withdrawl created a change in the high progesterone level existed in the anestrous buffaloes and initiated the follicular development by way of negative feed back mechanism (Takker *et al.*, 1983).

Use of gonadotrophic releasing hormone (GnRH, "Receptal", Hoechst) administered in this study as a single intramuscular injection induced estrous in 10 of the 20 treated animals (50 per cent) with a mean interval of 10.80±3 days. However a low conception rate of 40 percent was recorded consequent to first A.I. (Table). The results observed in this study are similar to those of Mujumdar (1989). Lower rate of estrous induction and conception as noticed in this study might be due to the short half life of the endogenously released FSH and LH.

Table: Induction of estrus and fertility using non-hormonal and hormonal drugs in post-partum anestrus buffaloes

		No.of	Anin ind	nals exhibited uced estrum	Interval betwee of treatment	en withdrawal and induction	Anima	is conceived
Group Treatment		animals		Number	of es	strus	No. percentage (over estrus induced	
			Percentage		Mean	Range	animals)	
1	'Fertivet' (300 mg tablet/day for 5 consecutive days)	20	16	80	21.00	13-26	14	87.50
H	'Prajana' (3 capsules / day / three consecutive days)	20	15	75	16.00	8.26	13	86.67
111	'Cyclomin-7' (2 bolus once in three days for two weeks)	20	11	55	23.63	12.43	9.	81.81
Ĩ	Lugol's lodine (Cervical paint once in every 3 days 3 times)	20	10	50	12.30	5-28	7	70.00
V	'Duraprogen' (consecutive days injected I/M)	20	16	80	7.43	5-11	12	75.00
VI	'Receptal' (Gn-RH) (5 ml injected I/M) as single dose	20	10	50	10.80	8-14	4	40.00
VII	control	20	6	30	30.16	20.37	4	66.66

- Aminudeen and Tanwar, R.K. (1988). Note on efficacy of clomiphene citrate in seasonal anoestrous in buffaloes. Indian J. Anim. Reprod. 9:66-67.
- Birade, H.S., Deopurkar, L.V., Deshpande, B.R. and Puntambokar, P.M. (1922). Comparative efficacy of Intrauterine iodine infusions in induction of estruous in anoestrous cows. Indian J. Anim. Reprod. 13:89-90.
- Deshpande, B.R., Hukeri, V.B., Velhankar, D.P and Sane. CR. (1976). Preliminary observations on "Fertivet" in induction of heat in ancestrous cows and buffalces. Indian Vet. J. 53:561
- Dugwekar, Y.G., Pangawkar, G.R. and Sharma, B.D. (1980). Induction of estrous in anoestrous cows treated with 'Fertivet'. Theriogenology. 13:126.
- Hukeri, V.B; Ansari, N.N. and Deshpande, B.R. (1979). 'Fertivet' trial on estrus induction and conception in anoestrous lactating buffaloes. Indian Vet.J. 56:958-61.
- Kadu, M.S. and Chede, S.A. (1992). Studies on hormonal and non-hormonal treatments for summer anoestrous in buffaloes. Indian J. Anim. Reprod. 13:168-170.
- Luktuke, S.N; Bhattacharya, A.R., Singh, S.K. and Khan, B.V. (1973). Studies on the observations in functional activity of ovaries in buffaloes, Indian Vet. J. 50:875
- Moustguard, J. (1969). Nutritive influences upon reproduction In: "Reproduction in Domestic Animals" H.H. cole and P.T. cupps. Ind.Ed. Academic Press, New York. 489.
- Mujumdar, K.A. (1989). Efficacy of 'Receptal' (GN-RH) treatment for various ovarian disorders in bovine. Indian J. Anim. Reprod. 10:183-184.
- Nagaraju, N; Reddy, V.S.C. Rao, A.S., Sharma, G.P., Reddy, G.V.N. and Reddy C.E. (1991) Studies on certain biochemical constituents of blood in cycling and true anoestrous buffaloes during post - partum period. Cheiron. 20:6-8.
- Naresh Chand (1974). A preliminary report on the effect of 'Prajana' on reproductive organs in immature mice. Indian Vet. J. 51:167-169.
- Patil, J.S.; Bugalia, N.S., Sinha, A.R.; Khanna, A.S. and Chopra S.C. (1983). Clinical efficacy of 'Prajana, lugol's paint and utero-Ovarian message in anestrous cows. Indian Vet. J. 60:1019-1020.
- Porwal, M.L., Saxena, H.K., Shrivastava, A.M. and Karandikar, G.W. (1976). Efficacy of different medicaments on anoestrous buffaloes. Indian Vet.J. 53:435-437.
- Rao, K.S. and Rao, A.R. (1984). Treatment of anoestruos in buffaloes with certain hormones. Indian Vet.J. 61:702-7.
- Reddy V.S.C; Sharma, G.P.; Raju, M.S. and Reddy, C.E. (1990). Effect of 'Clofert-vet' treatment in Post-partum anestrous crossbred cows and Murrah buffaloes. Indian J. Anim. Reprod. 11:75-76.
- Satishkumar (1986). Use of Tonophosphan (Sodium Salt of 4-Dimethylamin-2-Methyl Phenyl Phospharic Acid) in treatment of anoestrous buffaloes. Indian Vety. Med. J. 10(12):228-229.
- Satishkumar (1990). Restoration of normal cyclicity in postpartum anoestrous buffaloes. Indian J. Vety. Med. 10 (1):45-46.
- Singh, R.B.Akhtar, M.H Biswas, S.C And Yadava, R.P. (1978). A combined therapy of Tonophosphan and 'prepalin forte' in anoestrous heifers. Indian Vety.J. 552:157-160.
- Singh, L.P. and Singh, B.K. (1986). Studies on the incidence and treatment of anoestrum in desi cows. Indian Vety. J. 63 (11):961-962.
- Takkar, O.P., Singh, M and Varma, P.N. (1983). Progesterone levels vis-a-vis anoestrum in buffaloes concurrent with profile during stages of oestrous cycle. Indian J. Dairy Sci. 36(2) 4:125-128.
- Umesh, K.R(1992). Studies on certain blood constitutents of non-descript rural buffaloes during normal cyclic and postpartum anoestrus periods. M.V.Sc. Thesis submitted to A.P. Agri. Univ. Hyderabad, A.P.
- Varma, B. and Kharche, K.G (1983). Comparative efficacy of 'Fertivet' and lugol's iodine, in anoestrous buffaloes. Indian J. Dairy Sci. 36(2):218-210.

130

IJAR. 15(2), 1994; 131-133

# Reproductive Disorders in Relation To Fertility And Milk Production In Tharparkar Cows And Their Crosses

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## ABSTRACT

Post-partum observations following 1044 calvings by 340 Tharparkar cows and their crosses covering a period of 11 studied to were observe the vears reproductive and productive efficiency following various reproductive disorders. The incidence of various reproductive disorders in the herd was 38.64%. The occurrence of reproductive disorders was more in 3/4 HF X 1/4 Thp (45.30%) than in 1/2 HF X 1/2 Thp (38.48%) and Tharparkar (37.01%) cows. The total milk vield in the cows with repeat breeding, cystic ovary and anoestrus conditions was more because of longer lactation period, however, the dry period and calving interval were significantly more. The repeater and cystic cows took significantly more number of services per conception with longer service period. The lactation yield in all the cows which suffered from retaind placenta, metritis. cervicitis. still-birth, abortion. dystocia and prolapse of vagina was low with increase in lactation period, dry period, service period, number of inseminations required per conception and calving interval resulting into very heavy economic losses.

The pathological condition of reporductive organs causing infertility or sterility in cattle may adversly affect the productive and reproductive performances. A number of cross-breeding programme are in operation in the country for the rapid improvement of indignous cattle. The evidence of reproductive disorders is higher with increse in exotic inheritence in the indigenous breeds of cattle (Pandey and Desai, 1973). Information on the comparative reproductive performance of indigenous cattle and their crosses with exotic breeds in relation to the dairy

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economics is of great significance. Keeping this in view the present investigation was undertaken in Tharparkar (Thp), 1/2 HF X 1/2 Thp and 3/4 HF X 1/4 Thp cows to assess subsequent productive and reproductive performance consequent to various reproductive disorders.

### MATERIALS AND METHODS

The post-partum observations following 1044 calvings by 340 cows (160 Thp, 112 1/2 HF x 1/2 Thp and 68 3/4 HF x 1/4 Thp) maintained at Livestock Farm, JNKVV, Jabalpur covering a period of 11 years ending December, 1979 were recorded. The various reproductive disorders taken into consideration were anoestrus, repeat breeding, retained placenta, abortion, metritis, cervicitis, cystmic ovary, still birth, dystocia and prolapse of vagina. The incidence of still birth in Tharparkar, prolapse of vagina and cervicitis in 1/2 HF X 1/2 Thp and cystic ovary, cervicitis and still birth in 3/4 HF X 1/4 Thp cows was not noticed. The productive and reproductive traits studied were lactation yield, lactation period, dry period, services per conception, service period and calving interval. Significance of difference ('t' test) productive and reproductive between performance before and subsequent to various reproductive disorders was analysed (Snedecor and Cochran, 1967).

## **RESULTS AND DISCUSSION**

The overall incidence of various reproductive disorders in all the cows was 38.64% (anoestrus, 16.47%; repeat breeding, 9.77%; retained placenta, 4.78%; abortion, 2.29%; metritis, 1.89%; cervicitis, 1.05%; dystocia, 0.86%; still

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birth, 0.57%; cystic ovary, 0.57% and prolapse of vagina, 0.47%). The occurrence of reproductive disorders was more in 3/4 HF X 1/4 Thp (45.30%) as compared to 1/2 HF X 1/2 Thp (38.48%) and Thp (37.01%) groups. Iver *et al.*, (1992) reported higher incidence of anoestrus (30.36%). cystic ovary (1.42%) and cervicitis (1.54%) and lower incidence of metritis (0.08%) in cross bred cows.

In majority of the cows during anoestrus, repeat breeding and cystic ovaries the lactation yield showed an increase. The increase in lactation yield was attributed to delayed subsequent conception because of these maladies together with increase in the lactation period. On the other hand those cows which suffered from retained placenta, abortions, metritis, dystocia cervicitis, prolapse of vagina and still birth had comparatively low milk yield because of trauma and stress to the birth canal and to the animal due to these disorders.

More milk yield in a particular lactation period with decrease in overall productive life in anoestrus, repeat breeding and cystic cases were also reported by Hewett (1968) confirming the present results. The present findings also colloborates with the several workers (Rasbeck, 1964; Arthur, 1975; Kay, 1978 and Pandit, 1978) where the decline in lactation yield was reported in cows suffereing from retaind placenta, abortion, metritis and prolapse of vagina. Thus the prolonged lactation period with reduced per day yield together with enhanced unpaying dry period in the cows with reproductive abnormalities were the considerable factors in decreasing the crucial productive life.

The number of services per conception was decreased after abortions in Tharparkar and 1/2

HF X 1/2 Thp cows, the improvement in conception rate after abortion was also reported by Wijerathe (1971). In animals with the reproductive disorders (repeat breeding, retained placenta, metritis, dystocia, cystic ovary cervicitis, prolapse of vagina and still birth) the services per conception was increased. Dawson (1958) and Hewett (1968) reported low fertility rates in repeat breeding cows. Dutta and Dugwekar (1988) reported that the cows having retained placenta had subsequently a lower conception rate, Kay (1978) recorded 1.85 and 1.52 average number of services per conception in calvings associated with retained placenta and normal calvings, respectively, Arthur (1975) reported that prolapse of vagina in extreme conditions may lead to lowered fertility.

The service period was also increased subsequent to all the reproductive disorders in all the breeds excepting abortions in Tharparkar cows. Kay (1978) had also reported that in cows service period in normal calvings was lower compared to the calvings followed by retained placenta. The calving interval was also more in all the breeds of cows subsequent to all reproductive disorders excepting still birth. Gudi et al., (1969) reported that long calving intervals reduce the number of calves and overall production of milk. Choudhary et al., (1974) concluded that pathological conditions of uterus like retained placenta, abortion, dystocia, metritis, prolapse of vagina leads to delayed uterine involution and decrease reproductive efficiency by increasing calving interval:

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#### REFERENCES

- Arthur, G.H. (1975). Veterinary Reproduction and Obstetrics. 4th Ed. The Macmillan Publishing company Inc. New York.
- Choudhary, G., Agasti, M.K., Banerjee, G.C. and Ghosh, M.M. (1974). Studies on certain aspects of uterine involution in Holstein x Hariana female at first calving. Indian Vet. J. 51:395.

Dawson, F.L.M. (1958). Bovine cystic ovarian disease : An analysis of 48 cases. Brit. Vet. J. 114:96.

Dutta, J.C. and Dugwekar, Y.g. (1988). Treatment of retained foetal membranes in bovines. Indian Vet. J. 65:76-80.

- Mudi, A.K., Sohini, A.D. and Kudade, S.V. (1969). Observations on reproductive characters in Murrah buffaloes at Arrey milk colony Bombay. Indian Vet. J. 46:416.
- Hewett, C.D. (1968). A survey of the incidence of the repeat breeder cow in Sweden with reference to herd size, season, age and milk yield Brit. Vet. J. 124:342.

- Iyer, C.P.N., Nair, K.P., Sudarsnan, V., Madhavan, E., Mathai, E., Nair, M.S., Vijayakumar, V. and Joseph, M. (1922). Reproductive disorders of crossbred cows of Kerala. Indian J. Anim. Reprod. 13:65-68.
- Kay, R.M. (1978). The change in milk production, fertility and calf mortality associated with retained placenta or the birth of twin. Vet. Rec. 102:477.
- Pandey, H.S. and Desai, R.N. (1973). Incidence of abortion in cross-bred cows in heavy rainfall areas. Indian Vet. 50:521
- Pandit, R.K. (1978). Studies of vaginal and uterine prolapse in buffaloes. Ph.D. Dessert. Punjab Agricultural University, Ludhiana.
- Rasbech, N.O. (1964). Fertility and reproductive disorders of various species of farm live stock in Denmark. Brit. Vet.J. 120:415.

Snedecor, G.W. and Cochran, W.G. (1967). Statisctical methods. 6th Ed. The Iowa State University Press, U.S.A.

Wijeratne, W.V.S. (1,971). Population study of abortion in cattle with sepcial reference to genetic factors. Ani. Prod. 13:229.

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# Calcium and Phosphorus levels in Cyclic and True Anestrous Buffaloes in Fluorosis Endemic Areas

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## ABSTRACT

A field investigation was carried out to study the changes in the concentrations of serum calcium and phosphorus in ten each of normally cyclic and true anoestrous rural buffaloes maintained under high, medium and low levels of fluoride endemic and fluorosis free areas. Highly significant (P<0.01) differences were observed in the values of serum inorganic phosphorus concentration between the cyclic and acyclic buffaloes in all the three levels of fluoride endemic areas. A non-sigificant calcium levels were recorded in all the three fluoride areas studied in this investigation. Significant (P<0.05) differences in the levels of calcium and phosphorus ratio was recorded in ths study between the Oestrus and anoestrous periods in buffaloes maintained only under high fluorotic area but not under medium and low fluorotic areas.

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Reports on the levels of serum calcium and phosphorus concentration in cyclic and acyclic cows and buffaloes suffering with fluorine toxicosis are lacking. However, Majumdar *et al.*, (1943); Majumdar and Ray (1946) and Hoogstratten *et al.*, (1965) have noticed no significant change in the serum calcium levels in cattle exposed to varying concentrations of fluorids in their diet. Hypocalcaemic condition in cattle with fluorine toxicosis was also reported (Tusl and Slesinger, 1970; Kessabi *et al.*, 1985). While Jones (1972) and Mohiuddin and Reddy (1980) recorded elevated levels of serum calcium in fluorotic affected animals.

Normal or non-significant difference in the levels of inorganic phosphorus concentration was recorded in cattle with signs of severe fluorosis by Hoogstratten *et al.*, (1965) and Araya *et al.*, (1990). Whereas, Tusl and Sleisinger (1970) and Jones (1972) have reported elevated levels of serum inorganic phosphorus levels in cattle affected with severe fluorosis.

#### MATERIALS AND METHODS

Eighty pluriparous, non-descript rural buffaloes aged between 6 to 7 years and weighing about 300 kg were used for this investigation. Among these, sixty animals suffering with dental fluorosis were selected from three fluorotic endemic villages of Nalgonda district of Andhra Pradesh. Analysis report (Report, 1990) revealed that the average levels of fluorine in ground water of these villages were 11.50, 5.65 and 3.25 ppm, respectively. Based on the ground water fluoride levels, three zones were demarcated as high, medium and low fluorotic endemic areas, to study the effect of fluorosis on the cyclic and true anoestrous conditions in buffaloes. Twenty buffaloes were selected from fluorosis free area of Nalgonda district itself. All animals which were free from genital infections abnormalities were included in this or investigation and were catogorised into four groups.

Group A: Buffaloes from high fluorotic endemic area.

- Group B: Buffaloes from medium fluorotic endemic area.
- Group C: Buffaloes from low fluorotic endemic area.
- Group D: Buffaloes from fluorosis free area (Control group).

Each group consisted of 20 buffaloes out of which 10 were normal cycling and 10 anoestrous.

Part of the M.V.Sc thesis submitted by the first author to the A.P. Agricultural University, Hyderabad.

Samples of blood from jugular vein were collected twice in normal cycling buffaloes (i.3. on day of Oestrum and day 10 of the cycle) and only once during the anoestrus period of the animals in each group.

About 20 ml of blood was collected asoptically and the sera was stored at -20°C for further processing. The sera was used for the estimation of calcium and phosphorus by following the procedures described by Trinder (1960) and Varley (19'80) respectively.

## RESULTS AND DISCUSSION

Table 1 shows the levels of serum calcium and phosphorus in both the normal cyclic (day 'O' and 10) and anoestrous rural buffaloes maintained under three levels of fluoride affected areas. Corresponding levels of calcium and phosphorus in non-fluoride area are also depicted in the table.

In all the four groups studied (group 'A' 'B' 'C' and 'D') the serum calcium concentration in cyclic and acyclic buffaloes showed no significant difference between the three phases studied i.e., follicular, luteal and anoestrous. Reports on the levels of serum calcium concentration in cyclic and acyclic cows and buffaloes suffering with fluorine toxicosis are lacking. However, few authors like Majumdar et al., (1943); Majumdar and Ray (1946) and Hoogstratten et al., (1965) have noticed nosignificant change in the serum calcium concentration in cattle exposed to varying concentrations of fluorides in their diet few authors have reported hypocalcaemic condition in cattle with fluorine toxicosis (Tusl and Sleisinger, 1970; Hillman, 1979; Kessabi et al., 1985). Whereas, Jones (1972) and Mohiuddin and Reddy (1980) recorded elevated levels of serum calcium in fluorotic affected animals. The discrepancy in the reported values might be due to variation in the age of the animals, duration of feeding period, source of fluorine, physiological stress, general level of nutrition and the effectiveness of body defense mechanism.

Non-significant difference in the serum calcium levels observed in this study in cyclic and acyclic buffaloes ingesting fluorine between 3.25 to 11.50 ppm in drinking water might be due to continuous mobilization of calcium from the animals body reserves.

Significant differences in serum inorganic phosphorus concentration were recorded in this study between normal cyclic and anoestrus rural buffaloes ingesting various levels of fluoride in drinking water. However, differences inserum inorganic phosphorus levels in normal cyclic and acyclic buffaloes between three groups i.e., three levels of fluorosis and fluorosis free areas, were not significant. Reports on the levels of fluorine toxicity are lacking in the literature. However, few authors have reported normal or non-significant difference in the levels of inorganic phosphorus in cattle with signs of severe fluorosis (Majumdar et 1943; Majumdar and Ray, 1946; al. Hoogstratten et al., 1965 and Araya et al., 1990). Whereas, some researchers reported eleveted levels of serum inorganic phosphorus in fluorotic affected cattle (Tusl and Slesinger, 1970 and Jones, 1972). The observations found in this investigation and the findings of other workers in this field indicate that a minimum critical level of inorganic phosphorus seems to be necessary for the initiation and maintenance of ovarian activity. It also indicates the existence of a close relationship between serum inorganic phosphorus level and reproductive hormones. The role of phosphorus in the regulation of oestrus cycle has been well documented by several authors (Roberts, 1971; Arthur et al., 1982 and Kesrl, 1982). The findings of this investigation and the reports of other authors in this field indicate that arrest of ovarian activity is probably due to the marginal deficiency of phosphorus created by fluoride toxicity and the same may be sufficient to cause disturbance in the pituitary-ovarian axis, without manifestation of phosphorus deficiency symptoms and consequently increasing the duration for the onset of post-partum oestrus in the buffaloes.

The differences in the levels of calcium and phosphorus ratio recorded in this study between the follicular (oestrus day) and anoestrous period were found to be significant (P<0.05) in buffaloes maintained under high fluorotic area only but not under medium and low fluorotic areas. Reports of calcium and phosphorus ratio in fluorosis affected cattle are not available in the literature. Phosphorus as such reported to be not important but the ratio between calcium and phosphorus has been

Period	- High Fluorosis Area (Group A)			Medium Fluorosis Area (Group B)			Lo	w Fluorosis (Group C)	Area	Fluorosis Free Area (Group D)		
	calcium (mg/ 100ml)	Phosphorus (mg/ (100ml)	Ca:P	Calcium (mg/ (100ml)	Phosphorus (mg/ (100ml)	Ca:P	Calcium (mg/ (100ml)	Phosphorus (mg/ (100ml)	CA:P	Calcium (mg/ (100ml)	Phosphorus (mg/ (100ml)	Ca:P
Day of Oestrus	7.83±	8.27±	0.97±	8.22±	8.13±	1.09±	8.41±	8.64±	1.02±	10.32±	8.99±	1.15±
(Follicular phase)	0.50	0.47	0.08	0.68	0.52	0.15	0.84	0.51	0.14	0.95	0.60	0.07
Day 10 of the cycle <sup>®</sup>	7.35±	7.62±	1.00±	7.18±	7.34±	1.10±	7.95±	7.96±	1.02±	9.65±	8.01±	1.13±
(Luteal phase)	0.35	0.52	0.0 <b>7</b>	0.45	0.63	0.17	0.53	0.50	0.08	0.88	0.56	0.08
Anoestrous phase	7.06±	5.78±	1.26±	6:54±	6.28±	1.10±	7.83±	6.56±	1.21±	8.01±	6.65±	1.26±
	0.57	0.35	0.11	0.58	0.48	0.14	0.48	0.37	0.06	0.83	0.36	0.17

Table Showing the Mean Values of Serum Calcium and Phosphorus in Normal Cyclic and Anoestrous Buffaloes in High, Medium, Low And Fluorosis and Fluorosis Free Areas: stressed essential for the efficient reproductive processes to occur and the ratio between the two should between 1.5;1 and 2.3;1 in cows by Hignett (1959) and Morrow (1977). A moderately above than optimum calcium and phosphorus ratio of 2.13 was

found in this study in rural postpartum anoestrus buffaloes maintained especially in high fluoride area and the same might have been responsible for the production of acyclicity in postpartum buffaloes leading to delayed postpartum oestrus.

#### REFERENCES

- Araya, et al., O.:Witter, F.; Villa, A. and Ducom, C. (1990). Bovine Fluorosis following Volcanic activity in the Southern Andes. Vet. Rec., 126:641-642.
- Arthur, G.H.; Naokes, D.E. and Pearson, H (1982). Infertility in the cow : General consideration, anatomical,functional and managemental causes. In : "Veterinary Reproduction and obstetrics", 5th Edn. Balliere Tindall, London, 295-327.
- Hignett, S.L. (1959). Some nutritional and other interacting factors influencing the fertility of cettle Vet. Rec. 7.247-255.
- Hillman, D.; Bolenbaugh, D.L. and convey E.M. (1979). Hypothyroidism and anemia related to fluoride in Dairy cattle. J. Dairy Sci., 62:416-423.

Hoogstratten, B.; Leone, N.C.; Legrande Shupe J.; Greenwood, D.A. and Lieberman, J. (1965). Effect of Fluorides on hematopoietic system, liver and thyroid gland in cattle. J.Ame. Med. Assoc., 192 (1):112-118.

Jones, G.W. (1972). Fluorosis in a dairy herd Vet.Rec. 90:503-507.

- Keorl, C.L (1982) Nutrient requirements of ruminants in developing countries. International Feed stuffs institutes, Utah Agricultural Experimental Station, Laganutah, p.p.29.
- Kessabi, M.; Khouzaimi, M.; Braum, J.P. and Hamliari, 4(1985) Serum biochemical effects of fluoride incattle. Fluoride; 18:227.
- Majumdar, B.N. and Ray, S.N.(1946). Fluorine intoxication of cattle in India II. Effect of fluoros is on mineral metabolism. I.J. Vet.Sci. and Ani. Hus; 16(1-5) : 107-112.
- Majumdar, B.N.; Ray, S.N. and Sen, K.c. (1943). Fluorine intoxication of cattle in India, I.J. Vet.Sci.and Ani.Hus., 13(2):95-107.
- Mohjuddin, S.M. Vikram Reddy, M (1989). Haematological and biochemical studies on fluoride toxicity in sheep. I.V.J., 66:1089-1091.
- Morrow, D.A. (1977) Nutrition and reproductive relationship in Dairy cattle. First All India Symposium on Animal Reproduction at Punjab Agricultural University.

Report, (1990). Report of the Ground Water department Nalgonda, Govt. of Andhra Pradesh.

Roberts, S.J. (1971). Infertility in the cow. In : In ; Veterinary obstetrics and genital diseases, 2nd Ed. CBS publishers and Distributors, Delhi : pp 453-458.

Trinder, P. (1960) Estimation of serum calcium Analyst, 85889.

- Tusl, J. and Slesinger, L. (1970). Effect of Large doses of sodium fluoride on fluoride and minerals in blood serum, hair and other bovine tissues. Fluoride 3(4):188-191.
- Varley H. (1980). Estimation of inorganic phosphorus. In : Practical clinical biochemistry, 5th Ed. William Heineman Medical Books Ltd., London PP. 882-885.

# Studies on Productive & Reproductive Traits of Triple Cross Ongole Breed

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## ABSTRACT

Productive and reporductive performance of four genetic groups comprising of FBO, BFO, FJO and JFO were studied.

There was no significant difference among the four triple cross bred groups (FBO, BFO, FJO & JFO) in respect of gestation period, post partum heat, lactation length and peak yield. There were significant differences in respect of ages at first heat, conception and calving and lactation yield among triple crosses, However productive and reproductive performance of FJO in general was superior to that of other three groups.

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Information concerning productive and reporductive performance of Ongole breed was reported by Hussain *et al.*, (1981). Reproductive performance of half breds of Ongole X Brown Swiss was reported by Sharma *et al.*, (1986a) and that of Ongole X Freissian by Sharma *et al.*, (1986b), Deshmuk and Kaikini (1989) studied the levels of exotic inheritence in Jersey X Sahiwal Crossbreeds. A few parameters on reproductive performance of three breed cross breeds of Ongole was reported by Rao *et al.*, (1984). Keeping in view of the paucity of information on productive and reproductive performance of triple crossbred the present paper is being reported.

## MATERIALS AND METHODS

The present work was undertaken to study the comparative performance of MBO (1/2 Friesian X 1/4 Brown Swiss X 1/4 Ongole), BFO (1/2 Brown Swiss X 1/4 Friesian X 1/4 Ongole), FJO (1/2 Friesian X 1/4 Jersey X 1/4 Ongole) and JFO (1/2 Jersey X 1/4 Friesian X 1/4 Ongole) cross breds in relation to reproduction and production on 30 cattle of each genetic group.

For heat detection, the animals were teased both in the morning and evening by a teaser bull apart from observing heat symptoms visually and finally confirming by rectal palpation of genital organs.

Statistical analysis was done as described by Snedecor and Cochran (1967).

Breeding efficiency was calculated by using

the formula of Wilcox *et al.*, (1967) Viz.  $365 (N-1) \times 100$  where 'n' is the number of calvings <sup>D</sup>and 'D' is the number of days from first to last calving.

### **RESULTS AND DISCUSSION**

Mean and standard error of different productive and reproductive traits are presented in Table - 1.

## Age at first heat:

Average values for age at first heat among four genetic groups ranged from  $514.31\pm28.92$ in FJO to  $752.79\pm28.37$  days in JFO. The lowest age at first heat in FJO was significantly different from that of three other genetic groups (P>0.05)

## Age at first conception:

The differences in age at first conception among the triple crosses were significant (P>0.05), the lowest being 666.14 $\pm$ 27.77 days in FJO while the highest was 806.72 $\pm$ 42.47 days in BFO. Deshmukh and Kaikini (1989) reported the age at first fertile heat in 50% and 75% exhotic inheritence as 896.56 $\pm$ 34.38 and 924.18 $\pm$ 65.16 days respectively in Jersey X Sahiwal crossbreds which is slightly higher than the present findings.

## Number of services per conception:

The average number of services per conception ranged between 1.58±0.18 and

Table -	1 Reproductive	and Productive	Peformance of	Triple Cross	(75% Exotic
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SI. No.	Triat	FBO	BFO	FJO	JFO
1.	Age at first heat (days)	723.32±28.37ª	696.24±27.34ª	614.31±28.92 <sup>b</sup>	752.79±28.37ª
2.	Age of Interconception (days)	774.87±34.44 <sup>a</sup>	806.72±42.47ª	666.14±27.77 <sup>b</sup>	771.36±27.89ª
3.	Number of services	2.03±0.22 <sup>b</sup>	3.44±0.79ª	2.31±0.33⁵	1.58±0.18 <sup>b</sup>
4.	Age at 1st calving	1053.65±34.99ª	1083.72±42.42*	959.03±25.94 <sup>b</sup>	1054.09±28.56ª
5.	Gestation period	279.65±1.30°	272.92±2.43 <sup>a</sup>	275.15±1.58ª	276.15±2.50°
6.	Post partum heat	209.84±29.59ª	175.12±15.63°	175.24±16.54ª	190.45±20.13
7.	Breeding efficiency	75.76±2.0 <sup>d</sup>	79.46±3.95ª	92.46±1.98ª	87.14±2.06 <sup>b</sup>
8.	Lactation length (days)	287.23±14.51*	312.04±11.49°	321.55±4.20°	299.24±12.92ª
9.	Lactation yield in 300 days or less	1244.23±83.69 <sup>b</sup>	1543.84±125.94ª	1434.92±62.6ª	1206.71±61.22 <sup>b</sup>
10.	Peak yield (kgs.)	7.65±2.1ª	8.89±0.44ª	8.04±0.34ª	7.23±0.29ª
11.	Total lactation milk yield (kgs.)	1335.91±160.89 <sup>b</sup>	1668.13±160.86ª	1505.50°65.59 <sup>b</sup>	1292.76±72.85 <sup>b</sup>

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Common Superscripts do not differ significantly  $\frac{No.}{n}$  of bservations. 30.

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 $3.44\pm0.79$ . BFO had significantly higher number of services while the differences in 3 other group were non significant. Sharma *et al.*, (1988) reported the number of services per conception were 1.3, 2.53 and 2.9 for Jersey, HF and Brown Swiss half breds with Ongole.

## Age at first calving:

The average values of age at first calving ranged from 959.03±25.94 to 1083.72±42.42 days. The differences were significant (P>0.05). FJO had significantly earlier age at frist calving, while the three other groups had no significant differences. Sharma *et al.*, (1988) reported a range of 821.14 to 948.36 days for Jersey, HF and Brown swiss half breds of Ongole.

## **Gestation period:**

The differences in length of gestation period among the 4 genetic groups were statistically non significant. The average values of gestation period of the triple crosses were similar to that of Ongole and F1 cross breds.

#### Post partum heat:

The postpartum heat among 4 genetic groups ranged between 175.12 to 209.84 days and the differences were not significent.

## **Breeding efficiency:**

The differene in breeding efficiency among the 4 genetic groups were significant (P>0.05). The highest breeding efficiency was recorded in FJO with  $92.96\pm1.98$  followed by  $87.14\pm$ and  $79.46\pm2.00$  in FBO.

Among 4 genetic groups FJO had the highest breeding efficiency' with the desirable lowest averages for reproductive traits like age at first heat, age at first conception, number of services, age at first calving and service period.

## Lactation length:

The average values of lactation length ranged from about one month from 287.23 days and differences were not significant among the four genetic groups.

## Lactation yield in 300 days or less:

The average first lactation yield was 653.4±23.25 kgs to Ongole (Javaramakrishna, 1981) while the averages in the triple crosses were ranging from 1206.71±61.22 in JFO 1543.84±125.94 kgs in BFO and the differences among four genetic groups were significant (P>0.05).

Though the yield was maximum in BFO, the difference in milk yield between BFO and FJO were non-significant. The differences among the three genetic groups - FBO, FJO and JFO were also nonsignificant.

#### Peak Yield:

The average peak yield among the four genetic groups ranged from 7.32±0.29 to 8.89±0.44 and differences were non-significant.

### Total lactation milk yield:

JFO group had significantly highest total lactation milk yield of 1668.13±160.86 kgs while the differences in the three other groups were non-significant.

#### REFERENCES

- Hussain, S.M., Sharma, P.L.N., Jayaramakrishna, V., Rao., G.N., Ratna Rao, T.V. and Chennacharyudu, K., (1981). Proceedings of International seminar and show on Ongole cattle Guntur A.P., India. pp:31.
- Jayaramakrishna, V. (1981). Proceedins of International seminar and show on Ongole Cattle Guntur, A.P., India. pp:54.

Rao, K.N., Sivaiah, K. and Siva Prasad Rao, L (1984). Indian J. Anim. Sci. 54:201.

- Sharma, G.P., Eswara Reddy, C., Shankara Reddy, I., Satyanarayana, A. and Sriramamurthy, A., (1986)a. Indian Vet. J. 63 (II): 919-922.
- Sharma, G.P., Eswara Reddy, C., Kotilinga Reddy, Y., Satyanarayana.A. and Sriramamurthy, A., (1986)b. Ind. J. Dairy Sci., Vol. XXXIV (12) 453 - 55.

Sharma, G.P.; Krishnaiah, N.; Mallikarjuna Rao, T.K.V.V.; Sivaiah, K. and Srirema Murthy, A. (1988). "Studies on reproductive traits of Ongole oross breede". Indian J. Dairy Science. 41 (2):202-23

Snedecor, G.W. and Cochran, W.G. (1967) Statistical methods 6th Ed. Iowa State University Press, USA.

# Effect of Heat stress and Gonadotropic Hormone Treatment on Folliculogenesis

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## ABSTRACT

Healthy adult albino female rats were subjected to heat stress at 40.0±0.5°C daily for five days. A group of heat treated rats given simultaneously serum were genadotropin FSH and LH. Another group was treated with hormones alone. Based on histology follicles were divided into seven types and folliculogenesis was quantified. affected folliculogenesis. Heat stress Hormone treatment alone resulted in five in folliculogenesis. fold increase Gonadotropic hormone tends to counteract the adverse effects of heat stress of folliculogenesis but the super ovulatory response of gonadotropic hormone is not fully expressed under high ambient temperature.

-x---x----x-

Heat stress was reported to adversely affect the reproductive function of both males and females. In females cleavage of zygote (Dutt et al., 1959), maturation of oocyte and preovulatory changes in the follicle were considered to be the critical stages most sensitive to harmful effects of heat stress (Doney et al., 1973). Decreased secretion of luteinizing hormone (Tucker, 1982), increased secretion of prolactin and increased amount of cortisol, progesterone from the adrenals were some of the significant hormonal changes reported to occur subsequent to heat stress (Ponce et al ., 1981). The present investigation was taken up to study the effect of heat stress and gonadotropic hormone treatment on folliculogenesis.

#### MATERIALS AND METHODS

Healthy adult albino female rats aged 120±5 days were utilised for the study. They were maintained under hygienic condition at room temperature of 28±2°C and RH of 70 percent and fed with pellet feed and water ad libitum. Heat stress: BOD incubator with humidity control was used to subject the rats to heat stress. Rats in batches of four were kept in the closed chambers of the incubator whose temperature was maintained at 40.0±0.5° and RH of 70 percent. Rats were heat stressed for two hours daily for five days.

Hormone treatment: Serum gonadotropin FSH (Biochem Pharmaceuticals Industries) 10 IU was administered intraperitoneally and 48 hrs later Luteinizing hormone (Intervet International) 10 Iu was given subcutaneously. In heat stressed group serum gonadotropin was given on day 1 and Luteinozing hormone on day 3 of heat stress period.

Fifty seven rats utilised were divided into Group I (n = 16) Heat stress; Group II (n = 16) Hormone treatment (n = 16); Group III (n = 16) heat stress and hormone treatment and Group IV (n = 9) untreated control. Four rats in groups I, II and III were sacrificed on 5, 10, 15 and 25 day after the beginning of heat stress and / or hormone treatment. The control rats in group IV were sacrificed separately in batches of three. The ovaries were collected from the treated and control group and processed for histological study following standard procedure (Humason, 1979). Serial sections of the ovary were prepared. This paraffin sections at 100 microns interval were selected and stained with haematoxylin and eosin. On average 35 ovarian sections per period were analysed.

Folliculogenesis: Based on the size of the oocyte, size of the follicle, numbers of cells constituting the follicular envelope and on the histology of the follicle the folliculogenesis was classified into seven stages (Pederson and Peters, 1968). Number of normal follicles with oocyte were counted under each stage of folliculogenesis. The data were statistically analysed adopting standard procedures (Snedecor and Cochran, 1967)

#### RESULTS AND DISCUSSION

Effect of heat stress and / or hormone treatment on percentage of incidence of follicles in different stages of folliculogenesis is presented in table 1.

The incidence of preovulatory follicles (stage seven) was only 0.4±0.3 per cent on day 5 post heat stress which was significantly lower then 6.4±1.7 per cent of controls. Even on day 10 and day 15 the incidence was less, but by day 25 the incidence increased to 7.9±1.3 per cent. On the contrary incidence of stage one follicles increased highly significantly to 30.5±2.1 per cent on day 5 and to 57.0±1.9 per cent on day 10 and then declined to 22.0±2.0 per cent by day 25 post heat stress. Transit time for stage five follicles to grow to preovulatory stage seven was reported to be 10 days in mice (Pederson; 1970 and Numazawa and Kawashima, 1982) and in rat (Osman, 1985). Therefore, the low incidence of stage seven follicles in heat stressed rats could be due to the subtle subcellular damage in stage five follicles caused by heat stress. The significant increase in the incidence of stage one follicle could be due to the arrest of entry of follicles into the proliferative pool mediated possibly through hormonal disturbance (Tucker, 1982 and Cahill et al., 1984).

In hormone treated rats there was five fold increase in the incidence of follicles of stage seven on day 5 post treatment. The incidence then declined to near normal level by day 25. The percentage of incidence of stage one was only  $4.4\pm1.9$  per cent on day 5 which was significantly lower then the control value of  $15.3\pm2.5$  per cent. Similar trend was noticed In stage two also. These changes were characteristic of superovulatory response of the ovary to hormone treatment. Recruitment of follicles into profiferative pool and accelerated maturation of follicles to preovulatory stage were reported to depend on Gonadotropic hormone (Hirshfield and Depaola, 1981 and Greenwald and Siegel, 1982).

There was two fold increase in the incidence of stage seven on day 5 when heat stress and hormone treatment were done simultaneously. Incidence of stage one did not vary significantly from that of control on day 5 but on day 10 increased significantly to 38.7±3.3 percent. The via-media changes observed suggest the possibility that gonadotropic hormone counteract the adverse effect of heat stress. Conversely it can also be interpreted that the superovulatory effect of gonadotrophin was suppressed by heat stress.

The stages five, six and seven were catagorised as antral follicle and the stages one, two, three and four as preantral follicle. The percentage of incidence of antral follicle increased markedly to 60.0 per cent after hormone treatment in contrast to 32.0 percent of control animals on day 5 but declined to near normal level by day 10 post stress. When the heat stress and hormone treatment were given simultaneously the percentage of antral follicle increased to 37.0 by day 5 but declined to 26.0 by day 10 and was similar to heat stressed animal after day 15 (Fig 1). These changes confirm the adverse effects of heat stress and the compensatory effect of hormone treatment on Folliculogenesis.



Table I Effect of Heat Stress and / or hormone treatment on the percentage of follicles in different stages of Folliculogenesis

Treatment	Stages in Folliculogenesis									
Days	1	2	3	4	5	6	7			
Control Gr. I Heat	15.3±2.5	24.2±2.9	8.2±1.9	20.1±2.8	18.4±2.7	7.4±1.8	6.4±1.7			
Stress										
5	30.±2.1**	15.5±1.7**	6.5±1.1	24.6±1.9	17.0±0.6	5.5±1.1*	0.4±0.3**			
10	57.0±1.9**	14.7±1.4**	4.3±0.8	13.2±1.3*	7.8±1.0**	2.4±0.6**	0.6±0.3**			
15	36.4±2.1**	14.4±1.5**	5.6±0.9	24.8±1.8	14.4±1.5	2.2±0.6**	2.2±0.6**			
25	22.0±2.0**	8.2±1.3**	6.3±1.1	32.9±2.3	18.5±1.9	4.2±0.9**	7.9±1.3			
Gr. II Hormone										
treatment										
5	4.4±1.9**	9.3±2.8**	4:6±1.9	21.4±3.9	18.9±3.7	10.8±2.9*	30.6±4.4**			
10	20.6±2.9**	10.6±2.0**	7.9±1.8	19.3±2.6	18.3±2.3	8.4±1.8*	14.9±2.4**			
15	28.5±2.4**	10.3±1.6**	7.0±1.3	19.5±2.1	18.6±2.1	3.9±1.0**	11.8±1.7**			
25	15.3±2.1	15.8±2.1**	10.8±1.8	25.0±2.5	19.9±2.3	4.7±1.2**	8.5±1.6			
Gr III Heat Stress and	10105 C						. distance			
Hormone treatment							The State of the S			
5	12.1±2.7	19.5±3.3*	8.0±2.2	23.5±3.4	18.8±3.1	6.1±1.9	12.0±2.7**			
10	38.7±3.3**	6.6±1.7**	8.2±1.5	20.5±2.9	11.7±2.2	7.5±1.8	6.8±1.7			
15	32.0±3.1**	8.6±1.9**	10.4±2.0	20.8±3.0	17.9±2.0	4.0±1.3**	6.3±1.6			
25	20.9±2.5**	12.3±2.0**	9.5±1.8	25.6±2.7	24.4±2.7	3.5±1.1**	3.8±1.2**			

Values marked (\*\*) or (\*) are significantly different from control values of corresponding stage.

#### REFERENCES

- Cabill, L.P., Oldham, C.M. Cognie, Y., Revalult, J.P. and Mauleon P (1984) Season and Photoperiod effects on follicles and Atresia in the sheep ovary. Aust J. Biol. Sci., 37:71-77
- Doney, J.M., Gunn, R.G. and Griffiths. J.G. (1973) The effect of premating stress on the onset of oestrous and on ovulation rate in Scottish Black Face Ewes. J. Reprod. Fert. 35:381-384.
- Dutt, R.H. Ellington, E.F and Carlton, W.W. (1959) Fertilization rate and early embryo survival in sheared and unsheared ewes following exposure to elevated air temperature. J. Anim Sci. 18:1308-1318
- Greenwald, G.S. and Siegal, H.I. (1982) Is the first or second preovulatory surge of FSH responsible for follicular recruitment in the hamster Proc. Soc. Exp. Biol. Med. *170*:225-230
- Hirshfield, A.N. and Depaola, L.V. (1981) Effect of suppression of the surge of follicle stimulating hormone with procine follicular fluid on follicular development in the rat J. Endocr. 88:67-71

Humason, G.L. (1979) Animal tissue techniques. 4th Edn. W.H. Freeman and Company San Francis Co.

Numazawa, A and Kawashima, S (1982) Morphometric studies on ovarian follides and Corpora lutia during the oestrous cycle in the mouse. J. Reprod. Fert. 64:275-283

Osman, P. (1985) Rate and course of atresia during follicular development in the adult cyclic rat J. Reprod. Fert. 73:261-270

Pederson, T. (1970) Follicle Kinetics in the ovary of the cyclic mouse. Acta Endocr. 64:304-323

Pederson, T and Peters, H., (1968) Proposal for classification of oocytes and follides in the mouse ovary J. Reprod. Fert. 17:555-557

Ponce, R.H., Thatcher, W.W., Collier, R.J. and Wilcox, C.J. (1981) Hormonal responses of lactating dairy cattle to TRH and HCTH in a shade management system in a subtropical environment. Theriogenology, *16*:131-138
Snedecor, G.W. and Cochran, W.G. (1967) Statistical Methods, 6th Ed. Oxford and IBH Publication Co., Calcutta. Tucker, A.H. (1982) Seasonality in cattle Theriogenology, *17*:53-59

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# Presumptive Capacitation of Bull Spermatozoa Incubated in Simple Chemically Defined Media

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## ABSTRACT

Washed bull spermatozoa resuspended in minimal culture medium (MCM-PL), Tyrode's medium (T) and phosphate buffered Locke's medium (PBL) at 40 to 60 million/ml concentration were incubated at 37-38°C for 8 hours in a B.O.D. incubator and examined for hyperactivated motility, hyaluronidase activity and lectin induced agglutinability to determine acquisition of presumptive capacitation of bull spermatozoa. Spermatozoa incubated in T showed distinct hyperactivated motility reaching a peak at 3 hours of incubation. Sperm hyaluronidass activity increased significantly from 4 hours of incubation in T and 6 hours in MCM-PBL. Lectin induced agglutinability of the spermatozoa increased from 6 hours in T and 4 hours in MCM-PL. Out of the three media studied T was more effective for induction of presumptive capacitation of bullspermatozoa in vitro

Knowledge on the process of sperm capacitation helps successful application of *in vitro* fertilization and embryo transfer techniques. Capacitation involves in spermatozoan surface alterations resulting in hyperactivated motility (Barros, 1974), increased hyaluronidase activity (Triana *et al.*, (1980) and increased lectin induced agglutinability of the spermatozoa (Talbot and Franklin, 1978). The present investigation was carried out to determine the efficacy of three simple chemically defined media for induction of presumptive capacitation of bull spermatozoa *in vitro*.

## MATERIALS AND METHODS

Ten semen ejaculates from three Jersey breeding bulls of the Indo-Australian Cattle Breeding Project, Guwahati were used in the study. Immediately after collection 2 ml semen from each ejaculate was washed twice by centrifugation at 600 g for 5 minutes with 0.9% Nacl solution, divided into three parts and resuspended in 10 ml each of MCM—PL (Barros, 1974), T (Rogers and Yanagimachi, 1975), and PBL (Johnson and Hunter, 1972). Each sperm suspension was divided into five equal parts, transferred to 5 polypropelene tubes and incubated at 37 to 38°C for 8 hours in a B.O.D. incubator. The sperm concentration ranged from 40 to 60 million/ml.

Sperm hyperactivated motility (Yanagimachi, 1981) and progressive motility were estimated at 1 hour interval by examining 0.02 ml sperm suspension under phase contrast optics at X 675 magnification and expressed in percentage.

Sperm hyaluronidase activity was determined at 2 hour interval using a spectrophotometer (Spectronic 20) following the method of Rogers and Morton (1973) and expressed in unit.

Lectin induced agglutinability of the supermatozoa was determined at 2 hour interval using concanavalin A as per the method of Talbot and Franklin (1978) and expressed in litre (lowest concentration of concanavalin A producing distinct sperm agglutination).

## **RESULTS AND DISCUSSION**

Bull spermatozoa incubated in T showed distinct hyperactivated motility reaching a peak at 3 hours of incubation (Table 1). This indicated acquisition of presumptive capacitation of bull

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Hours of	MCM	PL		r	PBL			
incubation tion	Hyperacti- vated motility	Progressive motility	Hyperacti- vated motility	Progressive motility	Hyperacti- vated motility	Progressive motility		
0	12.40±0.03	53.82±0.18	29.25±0.16	40.50±0.07	-	32 90±0 33		
1	6.35±0.18	30.72±0.32	36.20±0.11	26.38±0.20		-		
2	-	-	39.00±0.40	14.35±0.23	-	-		
3			39.20±0.46	5.23±0.24	-	-		
4	-	-	28.30±0.66	-	-	_		
5	-	-	24.15±0.56	-	- 1	-		
6	-	-	13.77±0.50	· -	-	-		
7	-	-	7.06±0.45	-		-		
8	-	-	-					

Table 1:Hyperactivated and progressive motility (%) of incubated bulls permatozoa (n=10)!

Table 2: Hyaluronidass activity and lectin induced agglutinability of incubated bull spermatozoa (n=10).

	Hours of incubation	H	yaluronidase activi (unit)	ty		Lectin Induced agglutinability (titre)		
		MCM-PL	Т	PBL	MCM-PL	T ·	PBL	
	0	a 2.58±0.32	a 3.03±0.38	1.64±0.27	16.72±1.17	11.80±1.12	15.60±1.23	
	2	ab 3.49±0.44	ab 3.40±0.45	8 1.86±0.31	14.55±1.27	10.29±1.20	20.23±1.30	
	4	ab 3.75±0.46	bc 4.97±0.60	a 1.87±0.33	6.19±1.28	10.29±1.20	19.65±1.26	
0	6	bc 4.81±0.60	bc 5.27±0.64	b 3.64±0.46		9.27±1.24	_	
	8	5.67±0.75	c 5.64±0.68	b 3.97±0.49	111-12	9.27±1.24	_	

Means bearing similar superscripts in a column do not differ significantly.

spermatozoa within 3 hours of incubation in T, MCM-PL and PBL did not sustain sperm progressive motility at incubation. The capacitation inducing property of T might be attributed to its albumin component (Byrd et 1985). al., 1979; Meizel. Characteristical extremely vigorous movement of the hyperactivated bull spermatozoa was consistent that observed with by Agrawal and Vanha-Perttula (1987) in capacitated bull spermatozoa.

Significantly increased hyaluronidase activity of the bull spermatozoa observed at 4 hours of incubation in T as against 6 hours in MCM--PL and PBL (Table 2) evinced early acquisition of sperm capacitation in T. Release f substantial amount of hyaluronidase into the incubating medium as observed by Triana *et al.*, (1980) was a component of capacitation of bull spermatozoa. Early release of higher amount of hyaluronidase by bull spermatozoa incubated in T might be due to serum albumin incorporated in the medium (Rogers and Morton, 1973).

The increased lectin induced agglutinability of the bull spermatozoa as indicated by decreased litre observed at 6 hours of incubation in T and 4 hours in MCM—PL (Table 2) might be due to spermatozoan surface change associated with capacitation as observed in laboratory animals (Talbot and Franklin, 1978; Talbot and Chacon, 1981). complete loss of lectin induced agglutinability of the spermatozoa after 4 hours of incubation in MCM—PL and PBI could not be explained.

It was concluded that out of the three media studied T was more effective for induction of presumptive capacitation of bull spermatozoa *in vitro*.

#### REFERENCES

- Agrawal, Y. and Vanha-Perttula, T. (1987) : Effect of secretery particles in bovine seminal vesicle secretion on sperm motility and acrosome reaction. J. Reprod. Fert. 79:409-419.
- Barros, C. (1974) : Capacitation of mammalian spermatozoa. Physiology and Genetics of Reproduction Part B. Plenum Press; New York, pp. 3-24.
- Byrd, E.W., Goodeaux, LL, Pool S.H. and Godke, R.A. (1979) : Effect of bovine serum albumin and Ca<sup>±</sup> on capacitation of bovine spermatozoa. Therogenology 11:93.
- Johnson, W.L. and Hunter, A.G. (1972). Seminal antigens : their alteration in the genital tract of female rabbits and during partial in vitro capacitation with beta amylase and beta glucuronidase. Biol. Reprod. 7:332-340.
- Meizel, S. (1985) : Molecules that initiate or help stimulate the acrosome reaction by their Interactin with the mammalian sperm surface. Amer. J. Anat. 174:285-302.
- Rogers, B.J. and Morton, B.E. (1973) : The release of hyaluronidase from capacitating hamster spermatozoa. J. Reprod. Fert. 35:477-487.
- Rogers, B.J. and Yanagimachi, R. (1975). Retardation of guinea pig sperm acrosome reaction by glucose : the possible importance of pyruvate and lactate metabolism in capacitation and the acrosome reaction. Biol. Reprod. 13:568-575.
- Talbot, P. and Chacon, R. (1981) : Detection of modifications in the tail of capacitated guinea pig sperm using lectins. J. Exp. Zool. 216:435-444.
- Talbot, P. and Franklin, L.E. (1978) : Surface modification of guinea pig sperm during in vitro capacitation : an assessment usinglectin induced agglutination of living sperm. J. Exp. Zool. 203:1-14.
- Triana, L.R., Babcock, D.F., Lorton, S.P., First, N.L. and Lardy, N.A. (1980) : Release of acrosomal hyaluronidase follows increased membrane permeability to calcium in the presumptive capacitation sequence for spermatozoa of the bovine and other mammalian species. Biol. Reprod. 23:47-59.
- Yanagimachi, R. (1981) : Mechanisms of fertilization in mammals. Fertilization and Embryonic Development in Vitro. Plenum Press, New York and London, pp. 81-182.

146

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# Separation of Acrosome of Bull Spermatozoa using Detergents and assessment of its Protein concentration by Spectrophoto metric method

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## ABSTRACT

Semen samples collected from eight different crossbreed bulls were treated separately with two different detergents viz. 0.75 mM Cety trimethylammonium bromide (CTAB) and 0.025% Teepol xL to effect separation of acrossomes from the sperm-head. CTAB was found to be more efficacious in separating acrosomes than Teepol xL (98,125% vs 75,875%), But both detergents caused serious damage to the acrosomes which became shrunken. disintegrated. distorted or Spectro photometric method of protein estimation indicated significantly (P<=0.05) more protein concentration in the CTAB extracted acrosomal suspension (0.505 ±0.0042 mg/ml) than in Teepol xL treatment (0.450 et al., 0.0027 mg/ml).



--x---x----x-----

contain mannose, galactose. to fucose. glucosamine, galactosamine and sialic acid as major sugars and glutamic acid predominated among amino acids (Hartree and Srivastava, 1965). The lipoglycoprotein extracted from the acrosomal preparations of ram, bull and rabit spermatozoa was found to have proteolytic and hyaluronidase activities (Srivastava et al., 1965). There is no published report on the estimation of exact amount of protein concentration of the acrosomal preparation of bull semen was done. So, the present investigation was aimed to separate acrossomes from the sperm head of crossbreed bulls using two different types of detergents, so that their comparative efficacy could be assessed and also to estimate the protein content of those acrosomal preparations by way of a modified Spectro photometric method.

## MATERIALS AND METHODS

Equal volume, i.e. 3 ml of good quality fresh semen samples (ejaculates) obtained individually from eight different healthy crossbreed bulls (xHF) and the individual semen sample (3 ml) was washed thrice by using Sperm Ringer's solution to obtain washed bull spermatozoa. The alliquote was then resuspended in normal saline upto twice the volume of original whole semen and each sample was then divided into two equal parts. To effect detachment of acrosome from sperm head two different types of detergents viz., 0.75 Cetyltrimethylammonium bromide mM (CTAB-LOBA CHEMIE, Bombay-2) and 0.025% Teepol xL (Sodium salt of a secondary alkyl

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sulphate. National Organic Chemical Industries Ltd., Bombay-21) solutions were added separately to those two fractions of washed bull sperm suspensions corresponding to individual bull following the methods described by Hathaway and Hartree (1963) and Hartree and Srivastava (1965). Acrosomes thus separated (in the supernatant fluid) and the sperm cells (as sediment) were stained with Giemsa solution as per the method adopted by Hancock (1952) except that the slides were kept in the stain solution for 24 hours at 35°C. Comparisons of the effect of the two detergents on the morphological changes of acrosomes and also on the percentage of detachment of acrosomes from sperm heads were made after microscopical examination.

The concentrations of protein of the acrosomal suspensions obtained by two different types of detergent treatments were estimated Spectro photometrically following Lowry's method (Lowry et al., 1951) and as modified by Hartree (1972) whereby a linear relationship between the concentration of soluble protein and colour yield was achieved which enabled the estimation of as low as 15-110 u gm range of soluble protein. In the present study instead of three concentrations as depicted by Hartree (loc. cit.), five different known concentrations viz., 0.01, 0.05, 0.1, 0.15 and 0.2 mg/ml of Bovine Serum, Albumin solutions (BSA) were used as standard protein and their corresponding Optical densities (at 650 m/u have length) were plotted to obtain the linear 'Standard Curve'. The results obtained were analysed statistically as per the methods of Snedecor and Cochran (1967).

## **RESULTS AND DISCUSSIONS**

The results of microscopical observations of the effect of two types of detergent treatments on eight individual semen sample were represented in Table-1. Examination of stained smear of sedimented fraction evidenced that the great majority of acrosomes were dislodged from the head region of spermatozoa both by CTAB and Teepol xL treatment (Fig 1) when compared with acrosome intact sperm head of normal (untreated) semen sample. CTAB was sound to be more effective than Teepol xL treatment as on average 98.125% and 75.875% detachment of acrosomes respectively were recorded, but both the detergents caused serious damage to the tails. Observations made on stained preparations of acrosome alone (taking the supernatant fraction) indicated that both the detergents distorted and disintegrated the separated acrosomes as was evidenced by many heavily red stained (Giemsa) granules and very few morphologically intact acrosomes. These observations corroborated with the similar findings of Hartree and Srivastava (1965) in their experiment with ram spermatozoa.

The results of protein estimation os acrosomal suspensions which were extracted wither by 0.75 mM CTAB or 0.025% Teepol xL detergent treatment were also represented in Table-1. The mean values of concentration (mg/ml) of protein in acrosomal suspensions extracted by CTAB and Teepol xL were 0.505±0.0042 mg/ml and 0.450±0.0027 mg/ml respectively.

The result of protein estimation indicated that significantly (P<=0.05) higher quantities of protein/ml of acrosomal suspension was found in 0.75 mM CTAB treated semen sample than with 0.-025% Teepol xL treatmet. This was clearly because of the fact that treatment with CTAB had better efficacy in dislodging more numbers of acrosomes from the heads of spermatozoa as was evidenced by mcroscopical obsrvations. It could be suggested that a discrete unit, the acrosome, being dissociated from the anterior part of the sperm-head with the treatmet of alkaline detergents, ultimately became soluble in the process of protein estimation by Spectro photometric method. Further, it was believed that the amount of protein estimated by this method contained some aminoacids of which glutamic acid predominated (Hartree and Srivastava, 1965) and still some other proteolytic enzymes like hyaluronidase, acrosin, coronarpenetrating and zona lysin etc. which might brought about dispersal of cumulus oophorus and corona radiata (Srivastava et al., 1965).

Detergents used		Observations made on eight acrosomal suspensions							Mean ± SE	Condition of detached acrosomes	
•		1	2	3	4	5	6	7	8		
	Spermatozoa without acrosomes (%)	98	95	98	100	97	100	99	98	98.125	Mostly shrunken and distorted, few intact acrosomes
075 mM CTAB											
	Protein Conc. (mg/ml)	0.51	0.48	0.50	0.52	0.51	0.51	0.51	0.50	0.505 <sup>a</sup> ±0.0042	
	Spermatozoa without acrosomes (%)	72	78	76	80	73	76	71	81	75.875	Fragmented and disintegrated acrosomes; mainly heavily stained granules. Very scanty intact acrosomes.
0.025% Teepol xL	Protein Conc	0.44	0.45	0.45	0.46	0.45	0.45	0.44	0.46	Ó.450 <sup>b</sup> ±0.0027	

Tale 1: Effect of detergents on the separation of acrosome of bull spermatozoa and estimation of its protein concentration.

Means having different superscribes in the same column differ significantly

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Plate 1. Showing head of bull spermatozoa without acrosome by Giemsa stain

## REFERENCES

Hancock, J.L. (1952). The morphology of bull spermatozoa. J. Exp. Biol. 20:445.

- Hartree, EnF. and Srivastava, P.N. (1965). Chemical composition of the acrosomes of ram spermatozoa. J. Reprod. Fert. 9:47-60.
- Hartree, E.F. (1972). Determination of protein : A modification of the Lowry method that gives a linear photometric response. Analytical Biochem. 48:422-427.
- Hathaway, R.R. and Hartree, E.F. (1963). Observations on the mammalian acrosome : Experimental removal of acrosomes from ram and bull spermatozoa. J. Reprod. Fert. 5:225-232.
- Lowry, O.H.; Rosebrough, N.J.; Farr, A.L. and Randall, R.J. (1951). Biol. Chem. 193 : 265. Cited by Hartree, E.F. (1972). In "Determination of protein : A modofication of the Lowry method that gives a linear photometric response. Analitical Biochem. 48:422-427.

Snedecor, G.W. and Cochran, W.G. (1967). Statistical Methods. 6th ed. Oxford and IBH Publishing Company.

Srivastava, P.N.; Adams, C.E. and Hartree, E.F. (1965). Enzymic action of acrosomal preparations on the rabbit ovum *in vitro*. J. Reprod. Fert. 10:61-67.

# Histomorphometrical Studies of Testis and Epididymis in Indigenous Pigs

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## ABSTRACT

. The organisation of germinal epithelium with the appearance of spermatozoa was complete at the age of 180 days. There was no significant difference in the left and right epididymal tubule diameter. The diameter of the cauda epididymus was significantly higher than that of the caput and corpus. The hight of the epithelium was the highest in the cauda and the lowest in the caput upto 5 months of age however the reversal pattern was noticed at 6-8 months age. The lumen of epididymus revealed spermatozoa at 6 months of age.

Studies on the histological and histometrical aspects on the testes and epididymis in pigs are scanty. But reports on similar studies are available in exotic pigs (McFee and Eblen 1967; Wrober and Fallenbacher, 1974 and Thomas and Raja, 1980 a&b and Alirich *et al...*, 1983). As there is no literature on such studies in the indigenous pigs, the present work was carried out.

-x---x---x-

#### MATERIALS AND METHODS

A total of 43 indigenous male pigs of All India co-ordinated Research Project on Pigs, College of Veterianary Science, Tirupati were utilized for this study. The number of animals used in each group from 0 day to 8 months were 0 day-4 months, 1 to 3 months-24(8x3) animals, 4 to 8 months-15(3x5) animals. Tissues were collected from upper, middle and lower regions of testis and from caput, corpus and cauda regions of epididymus after open method of castration and fixed in 10% formal saline. These were processed by routine paraffin embedding method. Section of 5 micron thick were cut and stained by haematoxylin and eosin method.

After calibrating occular micrometer against the stagemicrometer at different magnifications,

the diameter of seminiferous tubules was measured under high power magnification (45x). A total of 10 round tubules were measured from each section of the region of both the teste and average was calculated.

Similarly the histomorphometry of the epididymis in 3 different regions was carried out for 2 animals in each age group. Here also the diameter and hieght of the epithelium and steriocilia of 10 round tubules was measured. Statistical analysis was carried out as per the procedure of Snedecor and Cochran (1967).

## RESULTS AND DISCUSSION

Testis: Islands of seminiferous cords were separated by multilayered interstium at birth. The cords revealed the marginal cells in a single layer along the basement membrane enclosing 2 to 3 centrally located gonocytes with average diameter of 10.2 $\pm$ 0.01  $\mu$ 

At 30 days of age, the intertitium was less with an increase in marginal cells and gonocytes. Degenerated spermatogonia were seen at 90 days of age (Fig 1). At 120 days multiple cell layers consisting of spermatogonia, primary spermatocytes and a few spermatids were seen without gonocytes. Some of these seminiferous cords showed lumen formation at this stage with a few cells resembling sertoli cells.

At 150 days there was progressive reduction in intertitium with corresponding increase in tubular diameter. The lumen formation was noticed in many seminiferous cords. All cell layers with spermatocytes and spermatids in various stages of elongation and maturation were noticed. However, the relative number of round and elongated spermatids were less indicating degeneration of primary spermatocytes and spermatids. These findings were akin to those of McFee and Eblen (1967) in Pitman Moore strain. Thomas and Raja (1980a) in Large White York Shire breed of pigs found the primary and secondary spermatocytes by 105-120 days of age unlike the present finding. In the present study organisation of germinal epithelium with appearance of spermatozoa was complete at 180 days of age(Fig2). But Thomas and Raja (1980a) reported the same at 150 days of age. However, Hauser *et al.* (1952) noticed the appearance of spermatozoa at the age of 175 days. The histology of testis between 6th and 8th month period did not show much difference except in increase in the diameter of seminiferous tubule.

The diameter of seminiferous cords/tubules at birth was  $28.23\pm0.53 \mu$  which increased to  $159.30\pm0.5 \mu$  at 8 months of age.Thomas and Raja (1980a) recorded higher values i.e 49.60  $\mu$  at birth and 215.14  $\mu$  at 8 months of age in Large White Yorkshire boars. The variation noticed might be due to the difference in breed characteristics. McFee and Eblen (1967) and Thomas and Raja (1980a) reported a significant correlation among the tubule diameter, testicular weight and body weight.

*Epididymis*: The diameter of the caput , corpus and cauda epididymis was observed to increase from  $64.97\pm0.26$ ,  $71.18\pm0.17$  and  $80.81\pm0.4$ microns respectively at birth to  $336.85\pm0.36$ ,  $373.50\pm0.38$  and  $395.33\pm0.33$  microns respectively at 8 months of age.

There was no significant difference in the epididymal diameter of left and right sides.

The diameter of cauda epididymis was significantly higher (P0.05) than that of the caput and corpus. The diameter of the epididymal tubes at caput, corpus and cauda regions differed significantly (P<0.05). These findings were similar to those of Thomas and Raja 1980b) in Large White Yorkshire boars. The hieght of the epididymal epithelium recorded were 15.08±0.10, 18.26±0.09 and '20.81±0.09 microns in caput, corpus and cauda regions of epididymal tubule respectively at birth which increased to 88.43±0.12, 71.06±0.06 and 60.20±0.06 microns respectively at 8 months age. The height of the epithelium was highest in the cauda and the lowest in caput upto 5th month, but at 6 to 8 month the epithelium showed a reversal pattern i.e. highest in caput and the lowest in the cauda. The epithelial height increased with corresponding increase in age. Similar findings were also recorded by Thomas and Raja (1980b) in Large White yorkshire boars.

The thickness of the intertitium between the epididymal tubules at all the regions reduced in size as the diameter of the tubule increased. The lumen of the epididymis revealed the spermatozoal at 6 months of age whose concentration at 7 months of age. Contrary to this, Thomas and Raja (1980a) found a few scattered sperms at 150 days in Large White yorkshire boars.



Testes-90 days. Note increased number of marginal cells and degenerating gonocytes in the centre of semiferous cords. M & E x 450.





Testes-180 days. Note different stages of spermatogenesis and numerous spermatids. B & E x 800

#### REFERENCES

- Allrich R D, Chisteson R K, Ford J J and Zimmerman D R 1983 Pubertal development of boar. Age related changes in testicular morphology and in vitro production of testosteron and estradiol-17B. Biology of Reproductiom 28(4):902-909.
- Hauser E R, Dickerson G E and Mayer D T 1952. Reproductive development and performance of inbred and crossbred boars. No Agricultural Experimental Station Research bulletin. Citied by McFee and Eblen, 1967 Testicular development in Miniature swine. Journal of Animal Science, 26(4):772-776.
- McFee A F and Eblen J R 1967 Testicular development on miniature swine. Journal of Animal Science 26(4):772-776.
- Snedecor G W and Cochran G (1967) Statistical methods VI edition, Oxford and IBH publishing Company, Calcutta. P.59-61.
- Thomas U P and Raja C K S V (1980a) Histological changes of testis during postnatal maturity in pigs. Kerala Journal of Veterinary Science 11(2):296-310.
- Thomas U P and Raja C K S V (1980b) Histological changes of epididymis during postnatal maturity in pigs. Kerala Journal of Veterinary Science 11(2):311-323.
- Wrobel K H and Fallenbacher E (1976) Reproductive and testicular characteristics of purebred and crossbred boars. Research report Agril Experimental station MP-90, 128-133. cited Animal Abstracts 45(7): Abstract No.3373.

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# Trans-Scrotal Circumference (TSC), Age, Body weight and Seminal Characters in Surti Bulls\*.

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## ABSTRACT

Studies were made on 30 Surti breed buffalo bulls from field semen banks pertaining to age, body weight, transcrotal circumference and seminal characters. The mean TSC value in 30 bulls studied was 36.281cms (range 29 to 45 cms). Correlations between:(a) body weight and TSC:0.986±0/031\*\* (P<0.01) and (b) TSC and sperm conc./ml x 106:0.962±0.052\*\* (P<0.01). Testis size was highly related to many of the satisfactory semen characters which were positively associated except the abnormal sperm % content which was negatively associated. Higher TSC value bulls produced better semen quality and good sperm morphology. Thus, TSC could be used to know the sperm production potential and for selecting superior bulls which is of enormous economic importance. \_\_\_\_\_x\_\_\_\_x\_\_\_\_\_

Various aspects of male reproductive efficiencny, though important have not received due attention. This has a great impact on husbandry of dairy stock which in turn govern the milk production and monetary gains. Our knowledge regarding fertility traits in indigenous bulls is poor. Current thinking on Breeding Soundness Examination concerns benefits of selecting bulls with a larger scrotal circumference for the purpose of augmentation of fertility and reduction of sub-fertility or infertility (Ott, 1987). The results of the field study made on TSC, Age, Body weight and Seminal characters in Surti breed buffalo bulls, have been presented in this paper.

#### MATERIALS AND METHODS

Sudies were made on 30 Surti breed buffalo bulls in the field, pertaining to their age, body weight; TSC(Scrotum+Testes) and seminal. characters. The ages of the bulls varied from 19 to 46 months. The body weight in kg was calculated according to Shastry and Thomas (1976). Testicular and Epididymal pathological conditions clinically detectable were ruled out by examination of the bulls under study prior to TSC measurement of scrotal circumference (CM) was made correctly at the widest point of scrotum (Rosenberger, 1979) with plasticized cloth tape that followed the contour of the scrotum more closely (Grove, 1968). Paired testes weight was calculated by multiple-Regression equation (using log value of testicular length, width and thickness) according to Ansari *et.al.* (1972).

Different seminal characters were studied according to standard semen laboratory procedures and techniques according to Herman and Madden (1953). Total solids in seminal plasma were measured according to Blom (1969) and initial fructose in the semen was estimated according to Mann (1964). Mean values of three field observations / bull made on all the parameters studied at different intervals were utilised for the purpose of study and statistical analysis (Snedecor and Cohran, (1971).

## **RESULTS AND DISCUSSION**

The trend of semen characters viz: sperm concentration, semen volume, sperm count and abnormal sperm count with reference to TSC have been presented in fig:2 to 5. It can be seen that testis size was highly related to many of the satisfactory semen characters. The mean TSC value of 30 Surti buffalo bulls studied was 36.281 cms (Range 29 to 45 cms). Most of the parameters studied were positively associated, with higher TSC measurement, higher was the

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Graph 3.TSC (cms) and Semen Volume (ml) in Surti buffalo bulls







Graph 5 :TSC(cms) and Abnormal Sperm(%) in Surti buffalo bulls



155

value of semen characters except in abnormal sperm per cent content which was negatively associated with having lower abnormal sperm % with higher TSC value. This meant that higher TSC value bulls produced better semen quality and good sperm morphology.

Highly significant correlation coefficient was observed for the study between Body weight and TSC =  $0.986\pm0.31^{**}$  (P<0.01) and TSC and sperm conc./ml x  $10^6:0.962\pm0.052^{**}$ (P<0.01), (fig 1 & 2). Thus, TSC could be used to know the sperm production potential (SPP) of the bulls because of the highly significant positive relationship with sperm productivity. It also meant that selection for higher TSC bulls will produce more semen of better quality and good sperm morphology. Factors like breed, age, body weight, season and year, rearing systems and nutrition can affect the testicular size and firmness.

Foote (1984) reviewed the subject of general evaluation of male reproductive capacity. The techniques of evaluation ranged from simple

physical of measurements to the use micro-surgery, histology, histochemistry, biochemistry, immunology ,electron microscopy and other analytical cytological techniques. In conclusion the size of the testis meet all the requirements. Garner (1984) reviewed the in-vitro methods for estimating the fertilizing capacity of sperm cells and summarized that though the newer laboratory technology was encouraging the goal of accurately predicting the fertilizing capacity from laboratory testes remained elusive. Hence, TSC measurement is very simple, clinically convenient, easily employed and deserves wide application in the field for estimation of male reproductive capacity. This method also offers scope for selection of young bulls. Hence, in current thinking on breeding soundness examination TSC of bull has emerged as one of the most important trait for general evaluation of male reprodutive capacity.

Acknowledgement Thanks are due to all the Gujarat Agriculture University faculty members and field staff who provided help for the study.

#### REFERENCES

- Ansari, M.M., Thomas, B.P., Shukla, R.K. and Menon, G.N. 1972. Association of testicular measurements with semen production in Surti buffalo bulls. Indian J. Anim. Sci. 42:465-467.
- Blom, E. 1969. Rapid refractometric determinetion of total solids in bull seminal plasma and its possible diagnostic value. Andrology 1(2):71.
- Foote, R.H. 1984. General evaluation of male reproductive capacity. 10th Internat. Congr. Anim, Reprod, & A.I., June, 10-14, 1984, Illionis, USA, Vol. IV : X 1-8.
- Garner, D.L. 1984. In vitro methods for estimating fertilizing capacity of sperm cells. 10th Internal, Congr. Anim. Reprod. & A.I. June, 10-14, 1984. Illionis, USA, Vol.IV, X:9-15.
- Grove, D. 1968. Andrologische Untersuchungen enZeburindern und Versuche Zur ic onserviseung von Rinder Samen Raumfemgzeraturen. Tierarztuchen Hochschule, Hannover, 1968.
- Herman, H.A. and Madden, F.W. (1953). Artificial Insemination of cattle. A Handbook and Laboratory Manual. Lucas Bros, Columbia.

Mann, T. 1964. The Biochemistry of semen and of the male reproductive tract. Methuen & Co. Ltd., London.

Ott, R.S. 1987. Current thinking on breeding soundness examination of bulls. Proc. Ann. Meet. Soc. for Theriogenology, Sep. 16-18, 1987, Austin, Texas, USA.

Rosenberger, G. 1979. Clinical examination of cattle. 2nd ed. Verlag Paul Parey, Hamburg.

Shastry, N.S.R. and Thomas, C.K. 1976. Farm Management 1st d. Vikash Pub. House, Pvt. Ltd. PP. 70-71.

Snedecor, G.W. and Cochran, W.G. 1971. Statistical Methods, 6th Ed. Iowa State Univ. Press, Ames, Iowa, USA.

## IJAR. 15(2), 1994; 157-158

## A note in Deep Freezing of Ram Semen

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Sheep is an important livestock species and they contribute greatly to the agrarian economy especially in the arid/semiarid and mountaneous areas where crop and dairy farming are not economical. Introducing artificial insemination in sheep with frozen semen of exotic outstanding rams will help in the devolopment of indegenous breeds of sheep for higher production of wool/hair and mutton.

Four Awassi rams in the age group of 1 year 8 months to 2 year 9 months having body weight of 55 to 75 kg belonging to Goat and sheep research and devolopment institute, Phaltan were taken for study. The semen collection was done every day between 9 to 9.30 a.m. by artificial vagina technique in the collection cup specially designed for the purpose without the use of rubber cones. This facilitated the collection of semen directly in the cup without wastage. The cup was held in plastic beaker having 35°C warm water to avoid thermal shock. Ten collections from each ram were taken over a period of 10 days. Observation on the semen ejaculate volume, sperm concentration, initial forward motility, live count, sperm abnormal count and acrosomal maintenance were recorded by using standard techniques. (Bhosrekar et al 1991).

The semen was then diluted in Tris-citric acid-fructose eggyolk dilutent (Bhosrekar et al

1991) in proportion of i:2 at room temperature. The diluted semen was then transferred to cold handling cabinet (5°C) along with the plastic beaker having water to avoid thermal shock. The cooling took 2.5 hours after which the semen was further diluted by pre-cooled portion of above dilutent containing 8 percent glycerol in three equal portions at the interval of 10 minutes, so that the final concentration of glycerol could be 4% and dilution rate could be 1:4. The semen was then packaged in mini (0.25ml) French straws. Equilibration period of 5 hours was given before the semen was frozen over liquid nitrogen vapour in wide mouth MVE320 container. The semen racks were held horizontally at the height of 20cm in the container for 8 minutes at the temperature of 80BC. The frozen semen was collected by pre-cooled tongs and placed on plastic goblets and was immersed in liquid nitrogen. The observation on mean values of seminal parameters and on freezability are given in table 1.

From the results it was seen that ram semen could be succesfully frozen and could be utilised for sheep improvement programme through artificial insemination.

The authors are thankful ro the President of BAIF and Director of Sheep & Goat Research & Devolopment Institute, Phaltanfor supporting this study.

### REFERENCE

Bhosrekar, M.R., Mokashi S.P., Purohit, J.R., Gokhale, S.B. and Mangurkar B.R. (1991). Studies on the effect of deep freezing and seasons on the leakage of aspartate amino transferase into extracellular medium and sperm morphology of Murrah buffalo bulls. Animal Reproduction Science 26, 219-226. IJAR. 15(2), 1994; 159

## **Ovarian Activity in Jakhrana Goats**

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Jakhrana, an important milch breed of goat is mostly confined to a small pocket in and around Jakhrana Village of Alwar district in Rajasthan. Ovulation rate and ovulation time are important reproductive traits in a breed. The information is equally important for undertaking embryo transfer programme in a livestock species. Such information is meagre in this breed. The aim of the present experiment was to study the ovarian profile in Jakhrana goats.

Twenty three adult cycling Jakhrana goats of different parties were used in the present experiment. The animals were maintained under semi-intensive management system comprising of 4-6 hrs daily grazing in forest area and feeding of adult ration @500/goat/day and green fodder *ad-libitum.* The oestrus was detected daily at 12 hrs interval by parading an aproned buck. The goats were subjected to exploratory laparotomy 72 hrs after the onest of oestrus. The ovaries were examined for the number of ovulation points/corpora lutea.

The mean ovulation rate was  $1.30\pm0.11$  (range:0-2). The right ovary was more active than

left ovary (21 VS 9). The oestrus symptoms were pronounced and typical. The mean duration of oestrus was 33.39±2.08 hrs (range:24-48 hrs). Large breed variations exist with regards to ovulation rates in goats (Rao and Bhattacharyya, 1980; Chemineau, 1983, AboulNaga, 1992, Greyling and Van Niekerk, 1990). It ranges from 1.5 to 4.0 in Indian goat breeds (Bhattacharyya and Prasad, 1974; Rao and Bhattacharyya, 1980). In the present experiment right ovary has been found more active than left ovary. This is in agreement with the findings of earlier workers in goats (Gonalez, 1977, Achunthan-Kutty and Raja, 1971). Theovulation rate is an important characteristic which reflects the number of eggs/ova liberated and eventually affects the number of kids born per doe (Prolificacy). An ovulatin rate of 1.30 recorded in Jakhrana goats is higher than a few exotic goat breeds (1.20 in Agora does by Shelton, 1960; 1.28 in Norwegian goats by Lyngset, 1968) and Marginally equal to 1.43 in Barbari does (Bhattacharyya and Prasad, 1974). It is substantially lower than Black Bengal goats (Rao and Bhattacharyya, 1980).

#### REFERENCES

- Aboul-Jaga, A.M. (1992). Genetics of Reproduction in Female oats. In : Pre Conference proceed, invited papers : V Intern Conf. on Goats, New Delhi, Vol II, part //:334-341.
- Achuthan-Kutty, A. and Raja, C.K.S.V. (1971) Studies on the incidence of early embryonic loss in goats based on abattoir speciman. Kerala JVet. Sci. 2:13-16.
- Bhattacharyya, N.K. and Prasad, S.P. (1974)- Ovulation Characteristics in nulliparous Barbari nannies. Proceed : 26th Intern Cong Physiological Sci, New Delhi 11:392.
- Chemineau, P. (1983) Effect on oestrus and ovulation of exposing Creole goats to the male at three times of the year. J. Reprod. Fertil. 67:65-72.
- Gonzalez, Stagnaro. C. (1977) Determination del momento de la ovulacion de cabras criollas en celo naturally sincronizado VI. Reunion de la Alpa La Habana Cuba.

Greyling, J.P.C. and Van Niekerk, C. (190) Ovulation in the Boer goat doe. Small Rumin. Res. 3:457-464.

Lyngset, O. (1968) Studies on reproduction in the goat. III The functional activity of the ovaries of the goat. Acta, Vet. Scand 9:268-276.

Rao, V.H. and Bhattacharyya, N.K. (1980) Ovulation in Black Bengal nanny goats. J. Reprod. Fertil. 58(1):67-69.

Shelton, M. (1960) Influence of the male goat on the initiation of oestrous cycling and ovulation of Angora does. Anim. Sci. 19(2):368-375.
# Therapeutic Efficiency of 'Hit-Rit' in Postpartum Anoestrous Buffaloes

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Prolonged postpartum anoestrous condition is invariably noticed in buffaloes resulting into long inter-calving periods and thus leading to reduced "calf-crop" and decreased milk production. The present concept of buffalo breeding is to produce a calf once in every 13 to 15 months for the purpose of achieving "white revolution".

Kodagali (1968), Rao and Murthy (1972), Deshpande (1983) and Naga Raju *et al* (1991) studied the true anoestrous condition in buffaloes under village conditions in different parts of the country and reported the incidence of anoestrum with quiescent ovaries ranging from 13.33 to 45 per cent. It was therefore, considered worthwhile to try 'HIT-RIT' (An indegenous drug manufactured by M/S Respal Pharma, Bangalore) in buffaloes suffering from post-partum anoestrus condition.

A total of 40 pluriparous buffaloes brought to the Ambulatory Veterinary Clinic, Mylardevpally. Ranga Reddy district, Andhra Pradesh were included in the trial. Ten buffaloes were kept as control animals. All the buffaloes were in apparent good health aged between 6 to 8 years and weighing about 300 to 400 kg. did not exhibit oestrus since a period of 6 to 9 months after parturition. Each experimental animal was orally administered 5 capsules of HIT-RIT daily for five consecutive days. The buffaloes of the control group were not given any treatment. All the animals were subjected to gynaeco-clinical examination daily and observations were recorded.

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Of the 40 buffaloes treated, 27 (67.5%) cycled, of which 17(63%) exhibited heat within 10 days after completion of 5 days treatment and 10(37%) shown oestrus between day 11 to 30. The tonicity of the uterus was found to be very pronounced in all the animals that exhibited heat. Of the 10 buffaloes of the control group only two animals manifested oestrus symptoms on their own accord during 30 days of observation period. However, the tonicity of the utrus in these animals was found to be not well pronounced.

Eighteen of the 27 buffaloes (66.7%) that exhibited heat after treatment with HIT-RIT were found to be pregnent with first insemination itself. Among the two buffaloes of the control group that were inseminated one animals was found to be pregnant.

This clinical trial has shown that HIT-RIT acts as an excellent oestrous inducer in post-partum anoestrous buffaloes and may prove to be very useful in solving the vexed problem like post-portum ancestrous condition in these animals.

#### REFERENCES

Deshpande, A.D. (1983). Aloes compound as an ovarian activator in Anoestrus buffaloes. Indian Vet.J. 60: 158-160.

Kodagali S.N. (1968). A note on reproductive disorders of farm animals in saurashtra. Indian J.Vet.Sci. 38:286-287.

Naga Raju, N., Reddy, V.S.C., Reddy, V.S., Rao, A.S., Reddy G.V.N, Sharma, G.P. and Reddy, C.E. (1991). Studies on certain biochemical constituents of blood in cycling and true Postpartum amoestrus buffaloes. Cherion, 20:1-3.

Rao, A.V.N. and Murthy, A.K. (1972), Studies on reproductive disorders in cows of Andhra Pradesh. Indian Vet. J. 49:61-68.

# IJAR. 15(2), 1994, 161

# Infertility in crossbred cows due to kinked cervix and its treatment

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In the ambulatory clinics and infertility treatment camps organised by the Banaskantha District Coop. Milk Producers' Union Ltd., most of the cases referred are related to infertility and reproductive problems in CB cows and buffaloes. The authors have observed a few cases of kinked (tortuous) cervix in CB cows. On rectal palpation, the cervix is felt like a firm cylindrical structure and almost at right angle to usual position of cervix. It was impossible to pass the insemination pipette even by careful manipulation. It was noted that all the CB cows with this defect were not conceiving after repeated services and were empty for a period ranging from one to two years or even more.

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It was also observed that these cows were cycling regularly and the body condition was quite good. On rectal examination, except for anomaly of the cervix, no other defect was noted except in one case which was uterus unicornis. It was noted that the development of follicle and CL was normal and there was no delayed ovulation in these animals.

A total of ten animals with kinked cervix were subjected to the following treatment:

On receipt of information about estrus, The animal when in mid estrus an intramuscular injection of oxytocin 10 I.U. (Neon make) was given. After about 4-5 minutes of time, the animal was inseminated as usual with a single dose of 0.5 ml. frozen semen. The insemination was done with due care but no special efforts were made to ensure passing of insemination pipette to the cranial part of cervix. The crossbred bull frozen semen used was from Sabarmati Ashram Gaushala, Bidaj.

All the ten cows became pregnant after 1-3 inseminations (average 1.8). It is reported that incidence of kinked cervix in heifers was about 0.1% (Roberts 1986). A few workers have reported successful pregnancies in such animals with intra peritonial insemination adjacent to ovary with graffian follicle. However, the prognosis was guarded and is seldom indicated. The present results are in agreement with the observation of Sandhu (1991) who has reported successful treatment in cows and buffaloes.

The probable mechanism of action of oxytocin is that it brings in strong contractions of reproductive tract and after some time, the smooth muscles become relaxed and allow sperm transport in larger numbers. It is also well established that for a successful pregnancy, the female reproductive tract has to be colonised by certain number of viable spermatozoa. This treatment which is cost effective and convenient is recommended for field use.

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### REFERENCES

Roberts S.J. 1986, Veterinary Obstetrics and Genital Diseases, Theirogenology, 3rd Ed. Published by author, Woodstock Vermount - USA PP 527.

Sandhu J.S. (1991) Proceedings of 9th National Symposium on recent biotechnological advances in animal reproduction held at Hissar - Haryana

### IJAR. 15(2), 1994; 162

## Efficacy of Gentamycin Sulphate Treatment of Repeat Breeding cows

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Repeat breeding is one of the major reproductive dis-orders in cows which causes great economic losses to the dairy owners. Although repeat breeding can be due to many factors endometritis is one of the major etiological factors under field conditions which is caused by in-correct handling of the semen, A.I. equipment and unhygenic conditions.

Gentamycin is poorly absorbed from the genital tract suggesting that the concentration in uterine lumen would remain high (Bretziaff; 1986). Over use of antibiotics in the bovine & equine uterus would lead to a shift in the nature of pathogenic bacteria or to the development of antibiotic resistant strains. Many pathogens isolated from the equine uterus were resisant to most antibiotics except the lesser used ones such as chloramphenicol or gentamycin (Whitmore & Anderson: 1986). There is paucity of data related to post breeding use of antibiotics. Keeping in view the above facts the present study was aimed to evaluate the efficacy of gentamycin in post breeding treatment of repeat breeding cows where common antibiotics had been tried by the veterinarians for the treatment endometritis.

Fifty three repeat breeding cows (50 cross bred and 3 Desi Cows) brought to Civil Veterinary Hospital Kalajhar (Sangrur) and Veterinary Polyclinic Patiala (Punjab) were given post A.I. treatment with gentamycin sulphate. The dose rate was 400-800 mg/animal. The volume of aquous infusion ranged between 50-80 ml. The infusion was made 2-18 hourspost A.I. Animals which did not repeat after first A.I. post treatment were checked per rectum for pregnancy diagnosis after 70-90 days.

Out of 53 cows treated 42 became pregnant. The encouraging efficacy of gentamycin in repeat breeders where common antibiotics had already been tried shows that there is more frequency of aerobic infections in the genitalia. Gentamycin is an aminoglycoside. Basic pharmacology and rsults of field trials with aminoglycosides both suggest that these drugs are poorly suited for intra-uterine treatment of anerobic infections (Olson & Mortimer; 1986). These studies show that there is more occurance of aerobic intrauterine infections in repeat breeding cows where common antibiotics have already been tried. However, further investigation by isolation and typing of bacteria in the laboratory is needed.

#### REFERENCES

- Bretziaff Katherine. N (1986) Factors of importance for disposition of antibiotics in the female genital tract. In Current Therapy in Theriogenology by David. A. Morrow; Philadelphia, WB Saunder's Co; 2nd Edition, Page 34-39.
- Olson Jersy D. and Mortimer Robert G. (1986) The Metrits-pyometra Complex. In Current Theraphy in Theriogenology by David A. Morrow Philadelphia, W.B. Saunder's Co; 2nd Edition, page 227-236.
- Whitmore Howard L. and Andeson Kevin L. (1986) possible adverse effects of antimicrobial treatment of uterine infections. In current Therapy in Theriogenology by David A. Morrow Philadelphia, W.B. aunder's Co : 2nd Edition, page 42-44.

IJAR. 15(2), 1994; 163

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### Fetal Maceration In a Jersy Cross Bred Cow - A Case Report

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The fetal maceration has been observed in cows following fetal death, regression of corpus luteum and failure of abortion to occur (Arthur, *et al* 1989). These authors have also opined that the treatment of fetal maceration with estrogens, corticoids and prostaglandins is unsuccessful. However, Jenkins, *et al* (1977) and Chetty *et al* (1986) reported success with prostaglandins, while Dhami *et al* (1988) with epidosin and Roberts (1986) suggested the use of estrogens in combination with oxytocin. The present report describes the successful use of estrogen and oxytocin in combination with local application of Povidone iodine in the treatment of fetal maceration in a jersy cross bred cow.

A Jersy cross bred cow in third lactation was presented at the Gynaecology Clinic, College of Veterinary Science and A.H., Anand, with history that the animal showed symptoms of straining at about third month of gestation. This was followed by a purulent vulval discharge since twenty days. The clinical examination of the animal revealed coconut size enlargement of right uterine horn with a feel of bony cripitation. Per vaginal examination revealed partly open cervical os and accumulation of purulant discharge in the anterior vagina and fornix. These features led to the diagnosis of fetal maceration.

The COW was administered diethvl stilboesterol 60 mg. intramuscular followed by 50 I.U. oxytocin, 24 hours later. Per vaginal examination 4 hours later, revealed the presence of fetal bones in the vagina and cervix. The bones were removed manually and providone iodine (Wokadine) 100 ml was administered intra uterine. On fourth day following the treatment, clinical examination revealed involution of the uterus and the vulval discharge was of mucoid nature with pus flakes. Wokadine 100 ml intra uterine was then repeated for three consequative days.

First oestrus after the treatment was exhibited 25 days later. Animal became pregnant with three consecutive A.I.

The successful outcome of this case suggest that if the fetal bones could be removed completly by inducing cervical relaxation with estrogens, contractions of estrogen sensitized uterus with oxytocin and then if the uterine infection is controlled, the prognosis could be favourable.

#### REFERENCES

- Arthur, G.H.; Noakes, D.E. and Pearson, H. (1989). Veterinary Reproduction and Obstetrics.6th Edn. Bailliere Tindall, London.
- Chetty, A.V.; Naidu, K.V.; Naidu, K.S. and Ramachandraih, S.V. (1986). Use of lutalyse in the expulsion of mummified foetus. Ind. J.Anim Reprod., 7(2):88-89.
- Dhami, A.J.; Panchal, M.T. and Parsani, R.R. (1988). Use of epidosin in expulsion of macerated and mummified foetus. Gujvet, 15(2):11-14.
- Jenkins, A.I.; Youngquist, B.S.; Elmore, R.G.; Kesler, D.J.; Garverick, H.A. and Bierschwale, D.J. (1977). Theriogenology, 4:251.
- Roberts, S.J. (1986). Veterinary Obstetrics and Genital Diseases. 3rd Edn. Edwards Brothers, Inc, Ann. Arbor, Mickign.

# Prepartum Eclampsia in a Pomeranian Crossbred Bitch

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A primiparous pomeranian crossbred bitch with history of 66 days pregnancy was presented to the veterinary polyclinic Patiala Punjab (Case No. 9623 Year 1992-93). The bitch was in recumbent state. The pupils were dilated. The tongue was protruding on one side and licking movements were absent. The respiratory rate was exagerated and typical panting was present. Muscular rigidity with occasional tremors were noticed. Hyperthermia with rectal temperature 105°F was recorded. Pervaginal examination revealed closed cervix with soft texture.

The treatment adopted included intravenous administration of 10% calcium gluconate (Dose 10ml). The influsion was made at a slow rate. Within fifteen minutes of the administration the muscular tremors and rigidity disappeared. The eye ball movement and reflexes were restored. The protruded tongue retracted with weak licking movements. The respiratory rate decreased and panting was absent. Half an hour after the influsion the bitch tried to sit and walk but the gait was ataxic. Keeping in view the progress of treatment another intravenous shot of 10% calcium gluconate (Dose 10ml) was given which lead to complete recovry of the bitch within one and a half hour after the first injection. Supportive treatment included intramuscular administration of Ampicilin (Dose 500mg) and Dexamethasone (Dose 10mg). The owner was advised to watch for whelping upto next day morning and if there was no progress cesarean was advised.

Next day the owner reported whelping of four dead pups early in the morning. The bitch was active. Respiration and rectal temperature were in normal range. Per abdominal examination revealed presence of two more pups. An intra-muscular injection of 20 units of oxytosin was given and the bitch delivered both the pups in 45 minutes. Both the pups were dead. The bitch was given antibiotic therapy (Ampicilin 500mg I/M) for seven days. The bitch was discharged and the owner was advised to give extra calcium-Phosphorus in her diet.

The failure of parturition to progress in this particular case at an average gestation period (63 days) might be due to low level of total or ionized serum calcium level which leads to ineffective uterine contractions. Several workers in the past have recommended the use of corticosteroids in the treatment and prevention of relapse. But this is without basis and may even be contraindicated as glucocortcoids decrease intestinal absorption and enhance renal excretion of calcium (Kaufman 1986). Oral administration of calcium or vitamin D supplements may be of benefit after the initial intravenous treatment. In this particular case dexamethasone was administered to induce whelping in conjuction with calcium therapy. However, studies related to induction of perturition in bitches at full term are lacking.

#### REFERENCES

Johnston Shirley D. (1986) parturition & dystocia in the bitch. In current Therapy in Theriogenology by David A. Morrow. Philadelphia WB Saunders Co; 2nd Edition page 500-501.

Kaufman Johanna (1986) Eclampsia in the Bitch. In current Therapy in Therio-genology by D.A. Morrow. Philadelphia WB Saunders Co; 2nd edition, page 511-512,

# Conjoined Twin Monstrosity In a Buffalo

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Conjoined twin monsters arise from a single ovum and are monozygotic. They are relatively more common in cow, sow, bitch and cat while, rare in other animals (Roberts '1971). A rare case of Dicephalus dipus hexabrachius Conjoined twin monster buffalo calf is recorded.

### CASE REPORT

About 71/2 years old local buffalo in her 2nd parity with dystokia was brought to the dispensary for treatment. The gestation period was complete and labour pains had started. Two hind limbs of the foetus were protruding out of the vagina. Pervaginal examination revealed a fully dilated cervix with a large sized pelvic portion of foetus, in the posterior longitudinal presentation, obliterating the birth canal. Caesarian Section was resorted to, when the manual attempts failed to relieve dystokia and a live conjoined monsters were removed, who died shortly after the operation. The monsters were fully developed and had a separate head and a neck each. Eyes and ears were normal. The two bodies fused with each other in a face to face position, starting from Sternal region (Sternopagus), progressing backwards up to the lower pelvic region (Ischiopagus). The fused pelvic region, however, had two separate tails (dicaudatus) with atresia ani. The monsters had a total of two hind limbs and six fore limbs (Fig.-1). It was a rare case of "Dicephalus dipus hexabrachius conjoined twin monster" as per the classification of Roberts (1971).

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Fig 1. Dicephalus dipus hexabrachius conjoined twin buffalo calf.

#### REFERENCES

Roberts, S.J. (1971): Veterinary Obstetrics and Genital diseases (Theriogenology) 2<sup>nd</sup>Edn. C.B.S. Publication.

# **Conjoined Twin Monstrosity In a Buffalo**

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