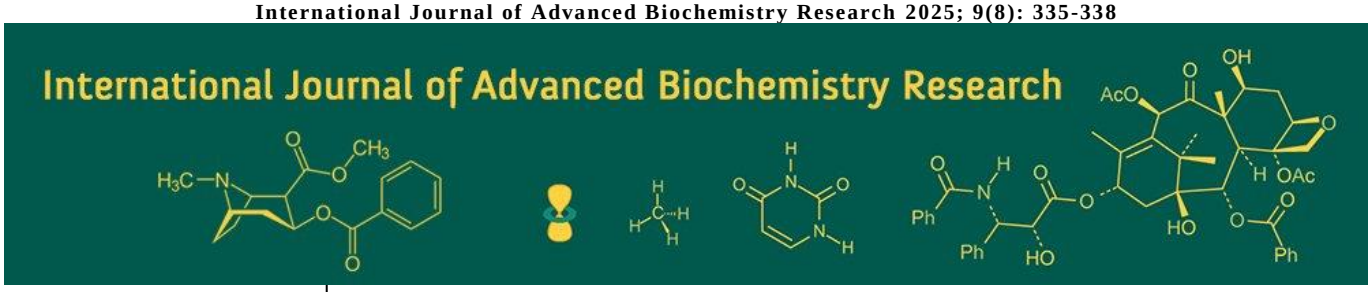


**ISSN Print:** 2617-4693
**ISSN Online:** 2617-4707
NAAS Rating (2025): 5.29
IJABR 2025; 9(8): 335-338
www.biochemjournal.com
Received: 09-06-2025
Accepted: 18-06-2025

**Yakkanti Nagendra Reddy**
Ph.D., Scholar, Department of ARGO, CVAS, Mannuthy, Kerala, India

**Magnus PK**
Assistant Professor, Department of ARGO, CVAS, Mannuthy, Kerala, India

**Jayakumar C**
Professor and Head, Department of ARGO, CVAS, Mannuthy, Kerala, India

**Abhilash RS**
Associate Professor, Department of ARGO, CVAS, Mannuthy, Kerala, India

**Lali F Anand**
Assistant Professor, Department of AGB, CVAS, Mannuthy, Kerala, India

**Manoj M**
Assistant Professor, LRS, Thiruvazhamkunnu, Palakkad, Kerala, India

**Syam Mohan KM**
Professor and Head (Rtd), ULF&FRDS, CVAS, Mannuthy, Kerala, India

**Corresponding Author:**
**Yakkanti Nagendra Reddy**
Ph.D. Scholar, Department of ARGO, CVAS, Mannuthy, Kerala, India

# Assessment of post retreival stress and ovarian complications in crossbred dairy cattle subjected to transvaginal oocyte retreival

**Yakkanti Nagendra Reddy, Magnus PK, Jayakumar C, Abhilash RS, Lali F Anand, Manoj M and Syam Mohan KM**

**DOI:** https://www.doi.org/10.33545/26174693.2025.v9.i8e.5175

**Abstract**
The objective of this study was to assess stress responses and ovarian complications following Transvaginal Oocyte Retrieval (TVOR) in crossbred dairy cattle. Eighteen animals were randomly assigned to three groups, all subjected to superstimulation protocol with porcine FSH (PFSH) prior to TVOR. Serum cortisol levels were measured at three time points: before superstimulation, immediately after TVOR, and on day seven Post-TVOR. Transrectal palpation and ultrasonography were performed on day seven to identify any ovarian complications. No significant difference in cortisol levels were observed among groups prior to superstimulation ($p>0.05$). However, animals in groups 2 and 3 exhibited significantly higher cortisol concentrations on the day of TVOR compared to group 1 ($p\leq0.05$), with no difference between groups 2 and 3. Cortisol values returned to baseline by day seven in all groups. Ovarian hardening and hypertrophy were observed in group 3, while luteal structures and blood-filled follicles were observed on ultrasonography in groups 1 and 2. No adhesions or major complications were detected in any group. These findings indicate that the cortisol rise after TVOR reflects an acute yet transient procedural stress response; the absence of major complications further confirms that TVOR is a safe, minimally invasive, and repeatable technique in superstimulated donor cattle.

**Keywords:** Superstimulation, follicle stimulating hormone, transvaginal oocyte retrieval, ultrasonography

## Introduction
Transvaginal Oocyte Retrieval (TVOR) when combined with in *vitro* embryo production (IVEP) enables the recovery of immature oocytes from antral follicles, significantly enhancing embryo output from live donors (Comizzoli *et al*., 2000) [3]. This is largely attributed due to the repeatability of the procedure, as oocyte collection can be performed up to twice per week over extended periods without adversely affecting the reproductive status of donor animals (Pieterse *et al*., 1991) [7]. TVOR is considered to be either a mildly invasive (Bols *et al*., 1995) [1] or a non-invasive technique (Qi *et al*., 2013) [8]. However, concerns primarily stem from the stress associated with repeated handling and restraint, which can lead to significant activation of the hypothalamic-pituitary-adrenal (HPA) axis, as evidenced by elevated cortisol concentrations following TVOR (Chastant-Maillard *et al*., 2003) [2]. Accordingly, serum cortisol is widely recognized as a reliable biomarker of stress and inflammation in cattle (Lomborg *et al*., 2008) [4]. In the present study, post-retrieval stress in donor animals was assessed by measuring serum cortisol levels at three time points: before super stimulation, immediately after TVOR (within 5-10 minutes), and on day seven post-TVOR. In addition, transrectal palpation and ultrasonographic examination were performed on day seven post-TVOR to evaluate potential ovarian complications resulting from the procedure.

## Materials and Methods
### Location and selection of experimental animals
The work was carried out at the University Livestock Farm and Fodder Research and Development Scheme (ULF & FRDS) Kerala Veterinary and Animal Sciences University,

Mannuthy, Thrissur. A total of 18 apparently healthy, normally cycling crossbred heifers or pluriparous dairy cows aged 2-6 years with an average milk yield of 4-10 litres/day, were selected. All animals were maintained under optimal management conditions. A detailed gynaecological examination was carried out in the selected animals to rule out reproductive pathologies such as cervisitis, endometritis, metritis cystic ovarian disease and ovario-bursal adhesions. Only animals free from such conditions were included in the study.

**Experimental design**

The selected animals were randomly assigned to three groups, each consisting of six cows. In group 1 (Hi-taper), superstimulation with tapering doses of pFSH at (100 mg, 60 mg and 40 mg). In group 2 (Int-cons), superstimulation with three injections of pFSH at a constant dose of 60 mg. In group 3 (Low-cons), super stimulation with three doses of pFSH at a constant dose of 40 mg at 12h intervals. In all three groups, TVOR was performed 48 h after the last pFSH injection. Blood samples were collected and estimated the serum cortisol levels just before the super stimulation protocol, on the day of TVOR and on the day seven post TVOR. To assess post-retrieval ovarian complications, transrectal examination and ultrasonographic examinations of both ovaries were performed on day seven with the aid of an ultrasound scanner employing a transvaginal convex probe of 5 MHz frequency (Honda HS- 2100, Honda Electronics Co., Ltd, Japan).

**Serological analysis**

Blood was collected from jugular vein in a clot activated tubes (Ultimate moulds and products, Thrissur, Kerala). Serum was separated by centrifuging the clotted blood at 5000 rpm for five minutes. Serum without any haemolysis was stored in a freezer at -20 ˚C for cortisol estimation. The serum cortisol levels were estimated using a Chemiluminescence immunoassay (CLIA) technique with a measuring range of 0.109-63.4 µg/dL. The intra-assay precision (within a single assay) and inter-assay precision (between different assay runs) was ranges from CV of 1.4-2.5%.

**Statistical analysis**

Serum cortisol concentrations were expressed as mean standard error (SE). Data were tested for normality using Shapiro-Wilk's test. As cortisol values were normally distributed, comparisons between groups were performed using one-way ANOVA, followed by Duncan's Multiple Range Test (DMRT) for post-hoc analysis. Period-wise comparison (before superstimulation, post-TVOR, and day 7 post-TVOR) was analysed using repeated measures ANOVA. All statistical analyses were conducted using SPSS software, version 24.0. Differences were considered statistically significant at $p \leq 0.05$ and highly significant at $p \leq 0.01$, following the guidelines of Snedecor and Cochran (1994) [9].

**Results**

The mean serum cortisol concentration (µg/dL) at the three evaluated time points for each group are presented in Table 1 and Figure 1. cortisol concentration (µg/dL) before super stimulation in groups 1, 2 and 3 were 0.75±0.22, 1.08±0.26 and 1.15±0.08, respectively with no significant difference among the groups ($p>0.05$). On the day of TVOR, mean cortisol concentrations (µg/dL) in groups 1, 2 and 3 were 1.94±0.35, 2.87±0.13 and 3.32±0.21, respectively. A significant ($p \leq 0.01$) increase in cortisol levels was observed in groups 2 and 3 compared to group 1, while there was no significant difference between groups 2 and 3 ($p>0.05$). On day seven post-TVOR, cortisol concentrations in groups 1, 2 and 3 were 0.60±0.22, 0.72±0.22 and 0.83±0.22, respectively, with no significant differences among the groups ($p>0.05$).

When cortisol concentrations were compared across time points within each group, there was a significant ($p \leq 0.01$) increase on the day of TVOR compared to both the pre-superstimulation and day seven post-TVOR levels.

In the present study, transrectal examination and ultrasonography were performed on day seven post TVOR to detect any ovarian complications resulting from the procedure. On transrectal examination ovarian hardening and hypertrophy were observed in group 3, while no ovarian adhesions were detected in any of the groups. Ultrasonographic evaluation revealed luteal structures (Figure 2), and blood-filled follicles (Figure 3) in groups 1and 2 but not in group 3.

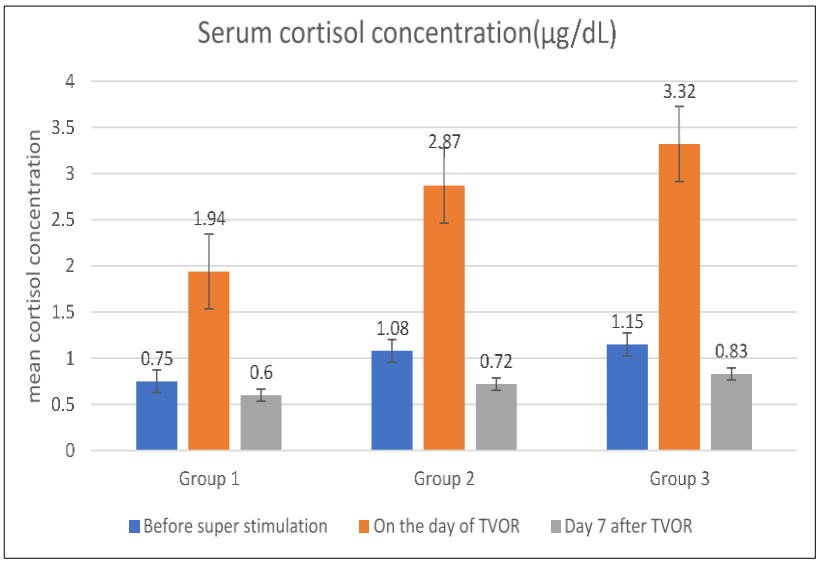


**Fig 1:** Mean serum cortisol concentration (µg/dL) before super stimulation, on the day of TVOR and on day 7 post TVOR

**Table 1:** Serum cortisol concentrations (µg/dL) at different time points in different treatment groups

| Period | Group 1 (N=6) | Group 2 (N=6) | Group 3 (N=6) | F-Value (P-Value) |
|---|---|---|---|---|
| Before super stimulation | $0.75^{B}$±0.22 | $1.08^{B}$±0.26 | $1.15^{B}$±0.08 | $1.115^{ns}$ (0.354) |
| On the day of TVOR | $1.94^{bA}$±0.35 | $2.87^{aA}$±0.13 | $3.32^{aA}$±0.21 | 8.014** (0.004) |
| Day 7 after TVOR | $0.60^{B}$±0.22 | $0.72^{B}$±0.22 | $0.83^{B}$±0.22 | $0.286^{ns}$ (0.755) |
| F-value(P-value) | 11.501**(0.003) | 26.336**(<0.001) | 46.703**(<0.001) | |

** Significant at 0.01 level; ns non-significant

Means having different small letter as superscript differ significantly within a row

Means having different capital letter as superscript differ significantly within a column

## Discussion

The present study aimed to evaluate the acute stress response and potential ovarian complications following TVOR in crossbred dairy cattle subjected to different superstimulation protocols. Serum cortisol concentrations served as a biomarker for stress, while transrectal palpation and ultrasonography provided insight into post-retrieval ovarian changes.

In the current study, serum cortisol levels were at basal levels prior to superstimulation. A significant increase was observed immediately post-TVOR in all groups, likely due to the combined effects of animal restraint, epidural anaesthesia, and the TVOR procedure itself. The highest post-retrieval cortisol concentrations recorded in in groups 2 and 3 compared to group 1 may be attributed to a greater number of small follicles, increasing the likelihood of the OPU needle piercing the ovarian cortex during follicle aspiration. This can result in localised haemorrhage, pain, and elevated stress responses. Conversely, group 1 with more medium-sized and large follicles, allowed easier aspiration by the operator, resulting in less haemorrhage, pain, and stress. Collected blood samples immediately post oocyte collection and observed a cortisol level of 2.53 µg/dL and within 15 min after collection, levels reached to 1.81 µg/dL. Similarly, petyim *et al.* (2007) [6] noticed cortisol levels rising from 1.66±0.56 µg/dL before TVOR to 4.82 µg/dL immediately following restraining, followed by a rapid decline after 10 min post-procedure. These findings support the view that cortisol levels rise sharply due to acute stress but normalize soon after the procedure. In the current study, blood samples were collected within 5-10 min post-TVOR and compared with samples taken before superstimulation and on day seven post-TVOR the trend of cortisol fluctuations observed in the present study underscores the transient nature of stress associated with TVOR procedures, with cortisol levels peaked on the day of oocyte retrieval and returned to baseline by day seven post TVOR. The significantly higher cortisol concentrations in the low-dose group (Group 3) likely reflect increased procedural difficulty and tissue trauma due to a predominance of small follicles. In contrast, lower cortisol levels in groups 1 and 2 affirm that optimal follicular stimulation not only improves oocyte yield but also minimizes procedural stress. Overall, these findings highlight the importance of effective superstimulation protocols and skilled handling to reduce
procedural stress during TVOR, thereby supporting procedural efficiency in IVEP programmes.

On transrectal examination, ovarian hardening and hypertrophy by thickening of ovarian tunica albuginea was noticed particularly in group 3. No ovarian adhesions were detected in any of the groups. The increased hardening is likely due to follicular punctures involving the ovarian cortex caused by the TVOR needle. Ultrasonographic findings included blood filled follicles, likely resulting from incomplete aspiration of dominant follicles, and luteal structures, which likely represented remnants of aspirated follicles. These changes are considered minor complications. Although TVOR has been associated with more severe lesions such as ovarian cysts, fibrosis, and scarring, which may negatively affect donor fertility (Oliveira *et al.*, 2019) [5], such gross pathological changes were not observed in the present study. These observations indicate that, when performed with proper technique and adequate animal preparation, TVOR procedures can be conducted safely without causing significant ovarian damage or compromising donor fertility. The absence of adhesions, cysts, or fibrosis in the present study supports the importance of operator expertise, careful handling, and appropriate anaesthesia in minimizing complications. Overall, the findings support the view that, under controlled conditions, TVOR is a low-risk and repeatable procedure suitable for use in IVEP programs in cattle.

## Conclusion

This study demonstrates that TVOR, when performed with optimized superstimulation protocols and skilled handling, induces only a transient stress response in donor cattle without causing major ovarian complications. Elevated serum cortisol levels observed immediately post-TVOR returned to baseline by day seven, indicating short-term procedural stress. Ovarian changes such as hardening, hypertrophy, and blood-filled follicles were minor and non-detrimental, particularly when effective stimulation protocols were employed. These results reaffirm that TVOR is a safe, repeatable, and minimally invasive procedure for oocyte collection in IVEP programs, provided it is conducted under controlled and humane conditions. Monitoring serum cortisol may serve as a useful indicator for assessing donor stress and ensuring welfare compliance in assisted reproductive practices.

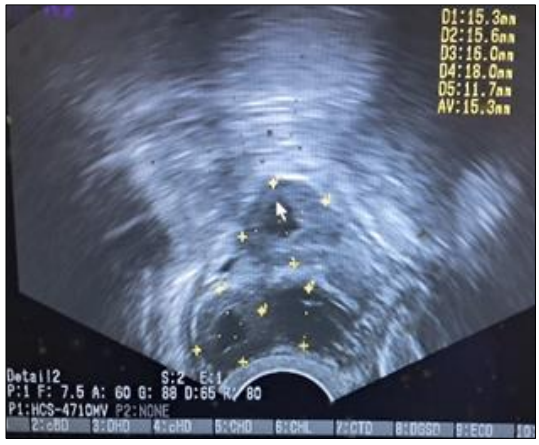


**Fig 2:** Ultrasonographic image showing luteal structures in donor ovary on day 7 post TVOR

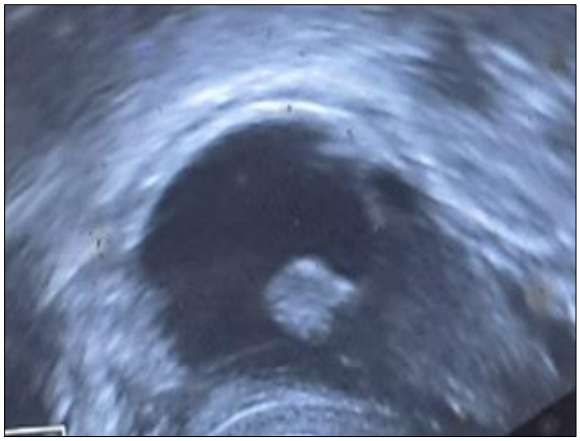

**Fig 3:** Ultrasonographic image showing blood filled follicle on day 7 post-TVOR

**References**


1. Bols PEJ, Vandenheede JMM, Van Soom A, de Kruif A. Transvaginal ovum pick-up (OPU) in the cow: a new disposable needle guidance system. Theriogenology. 1995;43(3):677-687.
2. Maillard CS, Quinton H, Lauffenburger J, Lefort CN, Richard C, Marchal J, *et al*. Consequences of transvaginal follicular puncture on well-being in cows. Reprod Fertil Dev. 2003;15(5):555-563.
3. Comizzoli P, Mermillod P, Mauget R. Reproductive biotechnologies for endangered mammalian species. Reprod Nutr Dev. 2000;40(5):493-504.
4. Lomborg SR, Nielsen LR, Heegaard PM, Jacobsen S. Acute phase proteins in cattle after exposure to complex stress. Vet Res Commun. 2008;32(7):575-582.
5. Oliveira LZ, de Barros CM, Monteiro MM, Rosa AS, Leal CL, Serapião RV, *et al*. Effects of ovarian super stimulation on oocyte recovery and embryo production in Holstein cows. Anim Reprod Sci. 2019;171:1-8.
6. Petyim S, Bage R, Madej A, Larsson B. Ovum pick-up in dairy heifers: does it affect animal well-being? Reprod Domest Anim. 2007;42(6):623-632.
7. Pieterse MC, Vos PLAM, Kruip TA, Willemse AH, Taverne MAM. Characteristics of bovine oestrous cycles during repeated transvaginal, ultrasound guided puncturing of follicles for ovum pick-up. Theriogenology. 1991;35(2):401-413.
8. Qi M, Yao Y, Ma H, Wang J, Zhao X, Liu L, *et al*. Transvaginal ultrasound guided ovum pick-up (OPU) in cattle. J Biomim Biomater Tissue Eng. 2013;18:1000118.
9. Snedecor GW, Cochran WG. Statistical methods. 8th Ed, Ames (IA): Iowa State University Press; 1994, p. 564.